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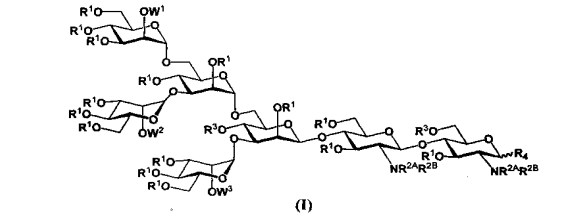
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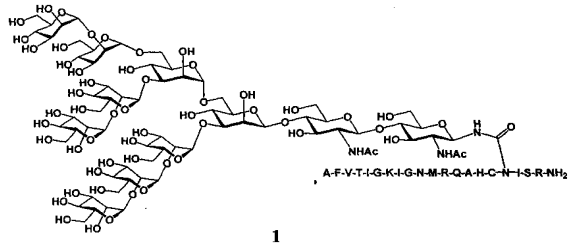
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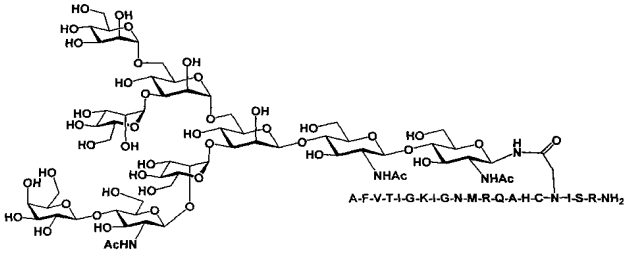
(54) Title: GP120 SPECIFIC ANTIGENS, CONJUGATES THEREOF, METHODS FOR THEIR PREPARATION AND USES THEREOF



(I)



1



2

(57) Abstract: The present invention provides compounds having formula (I): wherein R^1, R^{2A}, R^{2B}, R^3, R^4, W^1, W^2 and W^3 are as defined herein; and additionally provides methods for the synthesis thereof, compositions thereof, and methods of use thereof in the treatment of HIV, methods for the prevention of HIV, and methods for inducing HIV-specific antibodies in a subject, comprising administering to a subject in need thereof, an effective amount of any of the inventive compounds as disclosed herein, either in conjugated form or unconjugated and in combination with a suitable immunogenic carrier. In another aspect, the invention provides an antibody or antibody fragment which binds specifically to a gp 120 glycan or glycopeptide of the invention.

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GP120 SPECIFIC ANTIGENS, CONJUGATES THEREOF, METHODS FOR THEIR PREPARATION AND USES THEREOF

PRIORITY

[0001] This application claims priority to U.S. Provisional Application Nos.: 60/500,708, filed September 5, 2003; 60/500,161, filed September 4, 2003; and 60/430,822, filed December 3, 2002; each of the above applications is hereby incorporated by reference in its entirety.

GOVERNMENT SUPPORT

[0002] The invention was supported in part by Grant Nos.: BC020513 and BC022120 from the US Army (DOD) Breast Cancer Research Foundation. The U.S. government may have certain rights in this invention.

BACKGROUND OF THE INVENTION

[0003] Despite enormous scientific effort, the development of a vaccine against HIV has proven to be a largely elusive goal. There are several major factors complicating the creation of such vaccine.

[0004] One problem stems from a very low immunogenicity of the viral surface. Pairs of the envelope spike proteins (gp120 and gp41) form a trimer, inside of which much of the potentially antigenic surface of the unprocessed precursor protein (gp160) is buried. Moreover, the "outer" face of gp120 is extensively glycosylated (and therefore unavailable for peptide - recognizing antibodies), further complicating the problem.

[0005] Secondly, the mature envelope oligomer is itself a very weak antigen. Many explanations have been proposed to explain the unusually low antigenicity of the viral envelope spikes. The "glycan shield" concept implies that steric hindrance created by *N*-linked carbohydrates of gp120 prevents the immune system form generating antibodies with a broadly neutralizing action. Another hypothesis states that binding of neutralizing antibodies to the CD4 site of gp120 leads to conformational changes and is entropically disfavored, thereby allowing for HIV neutralization escape. It has also been suggested that a very strong initial immune response to gp160, which does not lead to broadly neutralizing antibody production

(vide supra) suppresses response to the mature oligomer, which is expressed in much lower concentrations.

[0006] In addition, extremely high degree and rate of viral variation provide a powerful mechanism for HIV to escape immune defense.

[0007] Accordingly, commonly utilized vaccine formulations have been unable to elicit a potent and broadly neutralizing antibody response. Administration of the whole virus in attenuated or inactivated form presents safety issues as well as the problem of low antigenicity. Immunization with a part of HIV DNA in a carrier is more promising, however it requires a very careful choice of the carrier virus. Also, low envelope antigenicity still remains a serious obstacle to the success of this method. A solution may lie in the use of artificial HIV antigens based on the epitopes of known broadly neutralizing antibodies. A highly focused immune response may be developed with this approach, potentially circumventing the problem of low antigenicity. The biggest challenge in this case is the design and synthesis of the antigens.

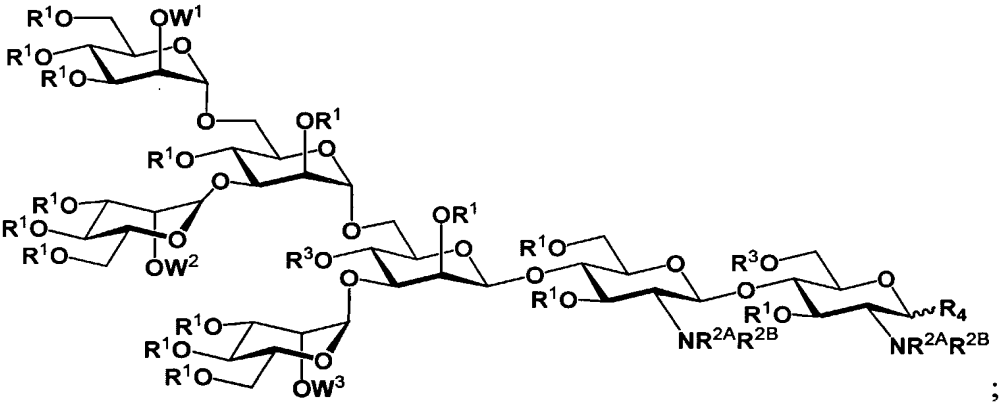
[0008] Gp120 surface carbohydrates can be seen as an attractive target for such design. There are a number of molecules that can efficiently bind to HIV envelope glycans. Among them, the dendritic cell receptor DC-SIGN has been demonstrated to recognize the internal tri-mannose segment of the N-linked oligosaccharides. A bacterial protein cyanovirin-N efficiently binds high-mannose type gp120 carbohydrates. Also, one of the most potent broadly neutralizing antibodies known to date, the 2g12, has been shown to have a carbohydrate epitope. Administering synthetic antigens containing one or more glycans on a part of gp120 peptide backbone or appropriately chosen linker system and further conjugated to an antigenic carrier could elicit strong immune response ultimately aimed at the real viral envelope. Some of the *N*-linked carbohydrates of gp120 appear to be conserved in most of HIV primary isolates. Since the glycans recognized by these molecules are located on the outer, "silent" face of the oligomer, they are easily accessible for antibody binding. Entropically disfavored interaction does not present a problem since the epitope does not overlap with the CD4-binding site. Finally, an extensive glycosylation of the envelope is an advantage, rather than a problem for such antigen design.

[0009] Accordingly, there remains a need for novel synthetic methods leading to the preparation of gp120 glycans and conjugates thereof, and their evaluation in immunologic and therapeutic studies.

SUMMARY OF THE INVENTION

[0010] In recognition of the need to provide access to synthetically unavailable gp120 glycans and glycoconjugates thereof, the present invention, in one aspect, provides novel gp120 glycans and glycoconjugates thereof, and methods for the synthesis and use thereof.

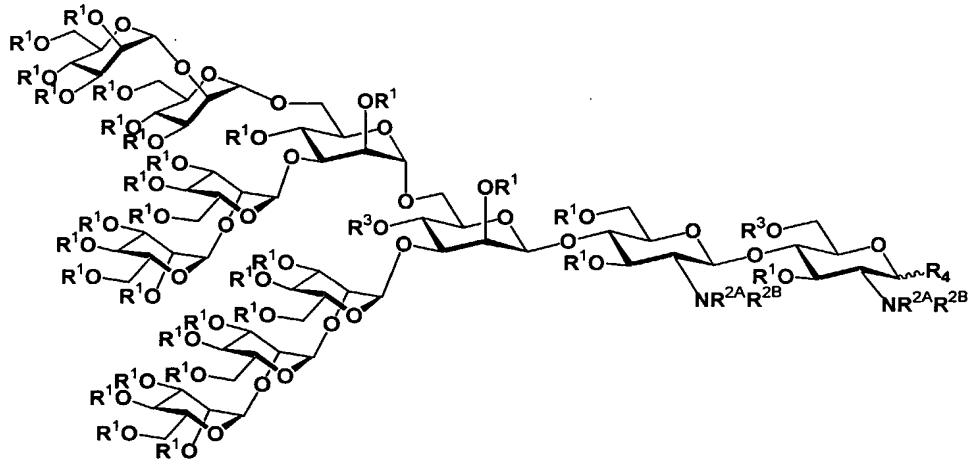
[0011] In one aspect, the present invention provides compounds having formula **(I):**



(I)

wherein R^1, R^{2A}, R^{2B}, R^3, R^4, W^1 , W^2 and W^3 are as defined herein.

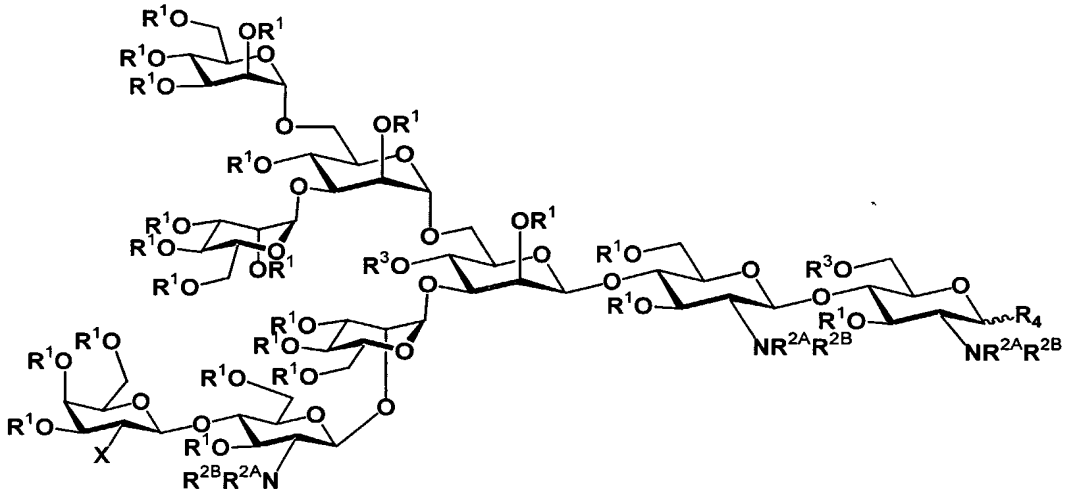
[0012] In certain embodiments, the invention provides a compound of formula **(II)** having the structure:



(II)

wherein R^1, R^{2A}, R^{2B}, R^3 and R^4 are as defined herein.

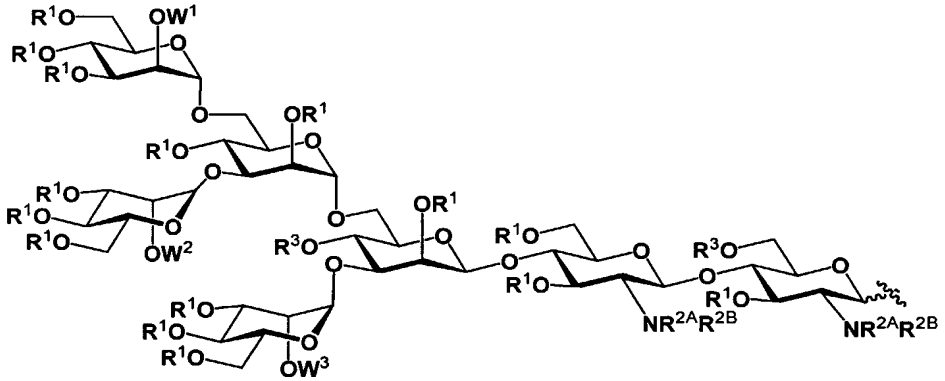
[0013] In certain embodiments, the invention provides a compound of formula **(III)** having the structure:



(III)

wherein X, R^1, R^{2A}, R^{2B}, R^3 and R^4 are as defined herein.

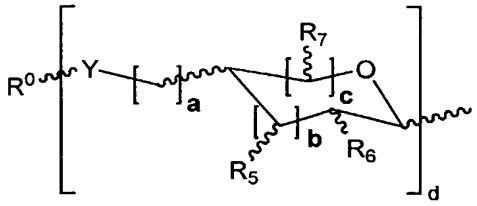
[0014] In another aspect, the invention provides multi-antigenic constructs comprising one or more carbohydrate domains having the structure:



wherein each occurrence of R^1 is independently hydrogen or an oxygen protecting group;

each occurrence of R^{2A} and R^{2B} is independently hydrogen or a nitrogen protecting group;

each occurrence of R^3 is independently hydrogen, a protecting group or a carbohydrate domain comprising a saccharide moiety having the structure:

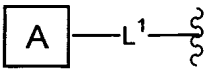


wherein Y is NH or O; wherein a, b and c are each independently 0, 1 or 2; d is an integer from 1-3; with the proviso that the d bracketed structure represents a furanose or pyranose moiety and the sum of b and c is 1 or 2; wherein R^0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein each occurrence of R^5, R^6 and R^7 is independently hydrogen, OH, OR^i, $NR^{ii}R^{iii}$, $NHCOR^i$, F, CH_2OH, CH_2OR^i, or a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of R^i, R^{ii} and R^{iii} is independently hydrogen, a protecting group, a sialic acid moiety, CHO, $COOR^{iv}$, or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group, or R^{ii} and R^{iii}, taken together with the nitrogen atom to which they are attached, form a substituted or unsubstituted heterocyclic or heteroaryl moiety; and wherein each occurrence of R^{iv} is independently H, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group;

W^1, W^2 and W^3 are independently optionally substituted mannose, galactose or lactosamine moieties;

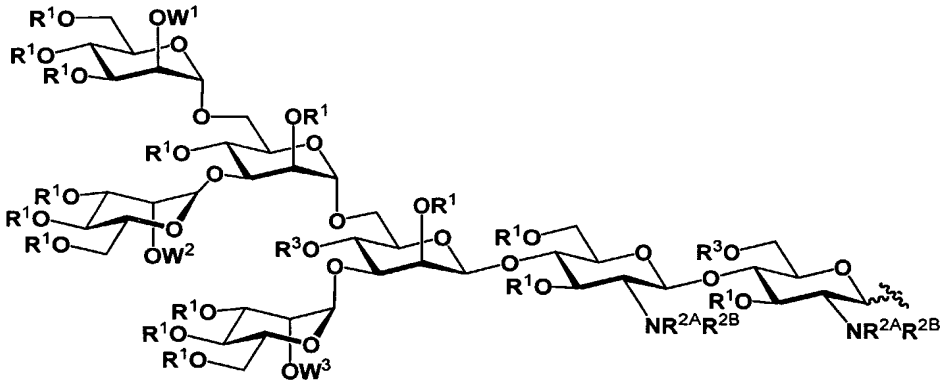
wherein each carbohydrate domain is independently covalently bound to a linker system, said linker system being a peptide or non-peptide nature, and wherein the linker system may be cyclic or acyclic.

[0015] In another aspect, the invention provides clustered glycoconjugates comprising a cyclic or acyclic backbone made up of two or more amino acids or other structural units, wherein one or more of said amino acids or structural units is/are independently substituted with a glycosidic moiety having the structure:

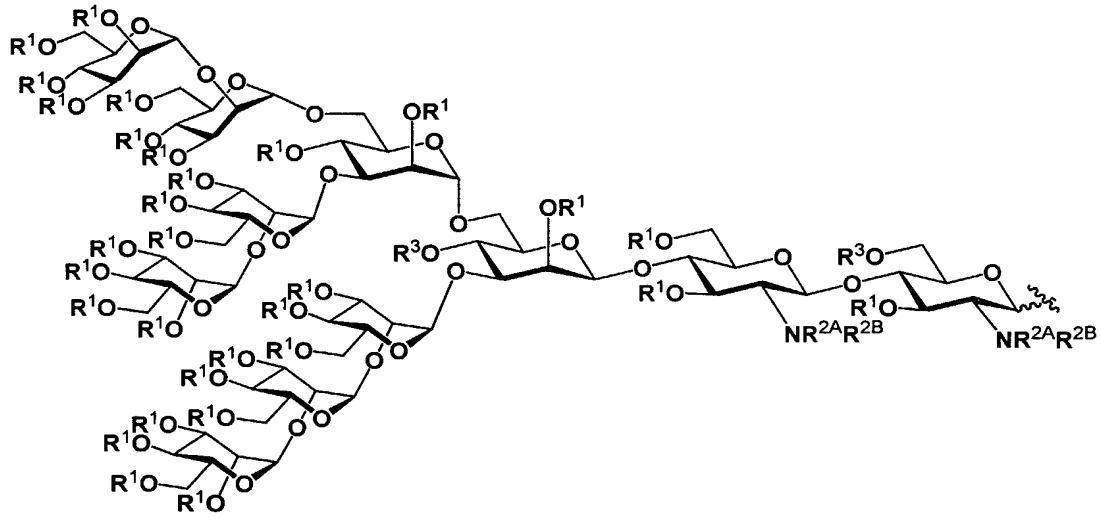


wherein each occurrence of L^1 is independently a substituted or unsubstituted, linear or branched, cyclic or acyclic, saturated or unsaturated aliphatic or heteroaliphatic moiety; and

and each occurrence of A is independently a carbohydrate domain having the structure:

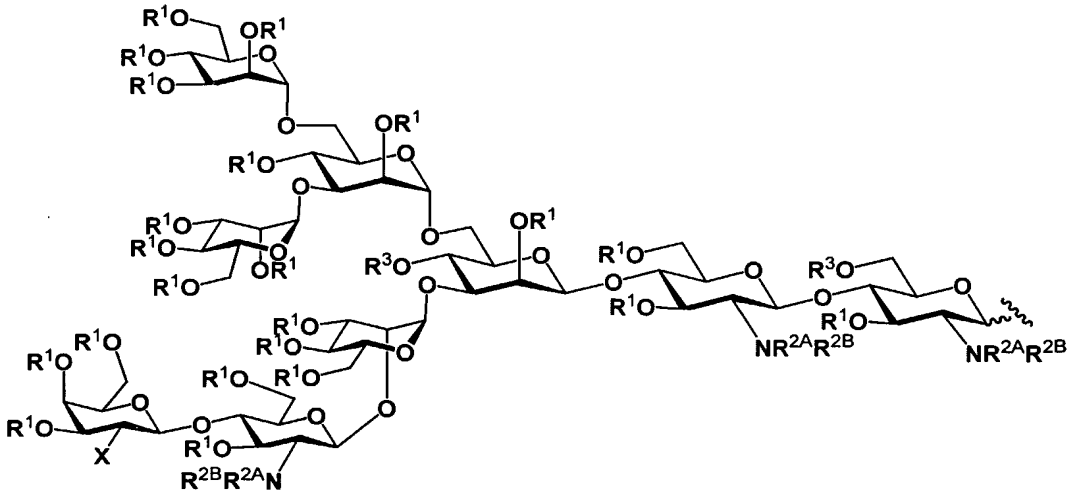


(I^{det})



(II^{det})

or

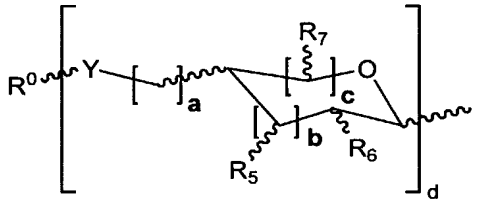


(III^{det})

wherein each occurrence of R^1 is independently hydrogen or an oxygen protecting group;

each occurrence of R^{2A} and R^{2B} is independently hydrogen or a nitrogen protecting group;

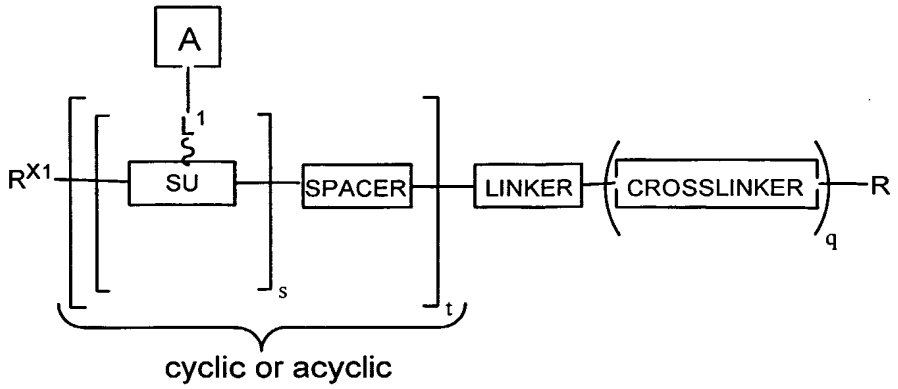
each occurrence of R^3 is independently hydrogen, a protecting group or a carbohydrate domain comprising a saccharide moiety having the structure:



wherein Y is NH or O; wherein a, b and c are each independently 0, 1 or 2; d is an integer from 1-3; with the proviso that the d bracketed structure represents a furanose or pyranose moiety and the sum of b and c is 1 or 2; wherein R^0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein each occurrence of R^5, R^6 and R^7 is independently hydrogen, OH, OR^i, $NR^{ii}R^{iii}$, $NHCOR^i$, F, CH_2OH, CH_2OR^i, or a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of R^i, R^{ii} and R^{iii} is independently hydrogen, a protecting group, a sialic acid moiety, CHO, $COOR^{iv}$, or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group, or R^{ii} and R^{iii}, taken together with the nitrogen atom to which they are attached, form a substituted or unsubstituted heterocyclic or heteroaryl moiety; and wherein each occurrence of R^{iv} is independently H, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group;

W^1, W^2 and W^3 are independently optionally substituted mannose, galactose or lactosamine moieties.

[0016] In another aspect, the invention encompasses clustered multi-antigenic constructs having the structure:



wherein q is 0 or 1;

each occurrence of s is independently an integer from 2-20;

t is an integer from 1-6;

R^{X1} is hydrogen, alkyl, acyl, aryl, heteroaryl, -alkyl(aryl), -alkyl(heteroaryl) or a nitrogen protecting group; or R^{X1} is covalently bound to a substituent on the last occurrence of the spacer, thereby forming a cyclic backbone;

R is hydrogen or an immunogenic carrier;

each occurrence of the structural unit SU is independently a substituted or unsubstituted aliphatic, heteroaliphatic, aryl, heteroaryl or peptidic moiety;

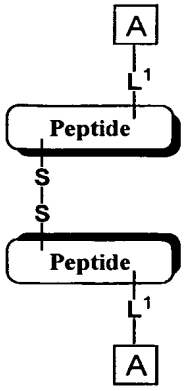
each occurrence of the spacer is independently a substituted or unsubstituted aliphatic, heteroaliphatic, aryl, heteroaryl or peptidic moiety;

the linker is either a free carboxylic acid, –O-, (carboxamido)alkyl carboxamide, MBS, primary carboxamide, mono- or dialkyl carboxamide, mono- or diarylcarboxamide, linear or branched chain (carboxy)alkyl carboxamide, linear or branched chain (alkoxycarbonyl)alkyl-carboxamide, linear or branched chain (carboxy)arylalkylcarboxamide, linear or branched chain (alkoxycarbonyl)alkylcarboxamide, an oligoester fragment comprising from 2 to about 20 hydroxy acyl residues, a peptidic fragment comprising from 2 to about 20 amino acyl residues, or a linear or branched chain alkyl or aryl carboxylic ester;

each occurrence of L^1 is independently a substituted or unsubstituted aliphatic or heteroaliphatic moiety; and

each occurrence of A is independently a carbohydrate domain of formula **(I^{det})**, **(II^{det})** or **(III^{det})**.

[0017] In yet another aspect, the invention provides dimeric glycopeptides having the structure:



wherein each peptide may be the same or different; each occurrence of L^1 may be the same or different and is as defined above; each occurrence of A is independently a carbohydrate domain of formula **(I^{det})**, **(II^{det})** or **(III^{det})**.

[0018] In another aspect, the invention provides compositions of any of the compounds, glycopeptides and/or constructs disclosed herein.

[0019] In another aspect, the invention provides an antibody or antibody fragment which is specific to one or more of the inventive gp120 glycans and/or glycoconjugates thereof described herein, said antibody being a purified polyclonal antibody or a monoclonal antibody.

[0020] In another aspect, the invention provides methods for the use thereof in the treatment of HIV, methods for the prevention of HIV, and methods for inducing antibodies in a subject, comprising administering to a subject in need thereof, an effective amount of any of the inventive compounds as disclosed herein, either in conjugated form or unconjugated and in combination with a suitable immunogenic carrier.

[0021] As detailed herein, in another aspect of the present invention, any of the inventive compounds may be conjugated to generate a glycoconjugate, and may be administered alone, with an immunogenic carrier (for example, a carrier protein, peptide or lipid), or with an immunological adjuvant or any combination thereof for the treatment of HIV infection and/or for preventing HIV infection, or may be administered alone or with an immunological adjuvant to induce antibodies in a subject.

[0022] In yet another aspect, the invention provides kits for conveniently and effectively carrying out the methods in accordance with the present invention.

DEFINITIONS

[0023] Certain compounds of the present invention, and definitions of specific functional groups are also described in more detail below. For purposes of this invention, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 75th Ed., inside cover, and specific functional groups are defined as described therein. Additionally, general principles of organic chemistry, as well as specific functional moieties and reactivity, are described in "Organic Chemistry", Thomas Sorrell, University Science Books, Sausalito: 1999, the entire contents of which are incorporated herein by reference.

[0024] It will be appreciated that the compounds, as described herein, may be substituted with any number of substituents or functional moieties. In general, the term "substituted" whether preceded by the term "optionally" or not, and substituents contained in formulas of this invention, refer to the replacement of hydrogen radicals in a given structure with the radical of a specified substituent. When more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the substituent may be either the same or different at every position unless otherwise indicated. As used herein, the term "substituted" is contemplated to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and nonaromatic substituents of organic compounds. For purposes of this invention, heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valencies of the heteroatoms. Furthermore, this invention is not intended to be limited in any manner by the permissible substituents of organic compounds. Combinations of substituents and variables envisioned by this invention are preferably those that result in the formation of stable compounds useful in the treatment and/or prevention of HIV, or in the inducement of antibodies, as described herein. The term "stable", as used herein, preferably refers to compounds which possess stability sufficient to allow manufacture and which maintain the integrity of

the compound for a sufficient period of time to be useful for the purposes detailed herein.

[0025] The term "aliphatic", as used herein, includes both saturated and unsaturated, straight chain (*i.e.*, unbranched) or branched aliphatic hydrocarbons, which are optionally substituted with one or more functional groups. As will be appreciated by one of ordinary skill in the art, "aliphatic" is intended herein to include, but is not limited to, alkyl, alkenyl, alkynyl moieties. Thus, as used herein, the term "alkyl" includes straight and branched alkyl groups. An analogous convention applies to other generic terms such as "alkenyl", "alkynyl" and the like. Furthermore, as used herein, the terms "alkyl", "alkenyl", "alkynyl" and the like encompass both substituted and unsubstituted groups. In certain embodiments, as used herein, "lower alkyl" is used to indicate those alkyl groups (cyclic, acyclic, substituted, unsubstituted, branched or unbranched) having 1-6 carbon atoms.

[0026] In certain embodiments, the alkyl, alkenyl and alkynyl groups employed in the invention contain 1-20 aliphatic carbon atoms. In certain other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-10 aliphatic carbon atoms. In yet other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-8 aliphatic carbon atoms. In still other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-6 aliphatic carbon atoms. In yet other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-4 carbon atoms. Illustrative aliphatic groups thus include, but are not limited to, for example, methyl, ethyl, n-propyl, isopropyl, allyl, n-butyl, sec-butyl, isobutyl, tert-butyl, n-pentyl, sec-pentyl, isopentyl, tert-pentyl, n-hexyl, sec-hexyl, moieties and the like, which again, may bear one or more substituents. Alkenyl groups include, but are not limited to, for example, ethenyl, propenyl, butenyl, 1-methyl-2-buten-l-yl, and the like. Representative alkynyl groups include, but are not limited to, ethynyl, 2-propynyl (propargyl), 1-propynyl and the like.

[0027] The term "alicyclic", as used herein, refers to compounds which combine the properties of aliphatic and cyclic compounds and include but are not limited to cyclic, or polycyclic aliphatic hydrocarbons and bridged cycloalkyl compounds, which are optionally substituted with one or more functional groups. As will be

appreciated by one of ordinary skill in the art, "alicyclic" is intended herein to include, but is not limited to, cycloalkyl, cycloalkenyl, and cycloalkynyl moieties, which are optionally substituted with one or more functional groups. Illustrative alicyclic groups thus include, but are not limited to, for example, cyclopropyl, -CH_2-cyclopropyl, cyclobutyl, -CH_2-cyclobutyl, cyclopentyl, -CH_2-cyclopentyl-n, cyclohexyl, -CH_2-cyclohexyl, cyclohexenylethyl, cyclohexanylethyl, norborbyl moieties and the like, which again, may bear one or more substituents.

[0028] The term "alkoxy" (or "alkyloxy"), or "thioalkyl" as used herein refers to an alkyl group, as previously defined, attached to the parent molecular moiety through an oxygen atom or through a sulfur atom. In certain embodiments, the alkyl group contains 1-20 aliphatic carbon atoms. In certain other embodiments, the alkyl group contains 1-10 aliphatic carbon atoms. In yet other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-8 aliphatic carbon atoms. In still other embodiments, the alkyl group contains 1-6 aliphatic carbon atoms. In yet other embodiments, the alkyl group contains 1-4 aliphatic carbon atoms. Examples of alkoxy, include but are not limited to, methoxy, ethoxy, propoxy, isopropoxy, n-butoxy, tert-butoxy, neopentoxy and n-hexoxy. Examples of thioalkyl include, but are not limited to, methylthio, ethylthio, propylthio, isopropylthio, n-butylthio, and the like.

[0029] The term "alkylamino" refers to a group having the structure -NHR'wherein R' is alkyl, as defined herein. The term "aminoalkyl" refers to a group having the structure NH_2R'-, wherein R' is alkyl, as defined herein. In certain embodiments, the alkyl group contains 1-20 aliphatic carbon atoms. In certain other embodiments, the alkyl group contains 1-10 aliphatic carbon atoms. In yet other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-8 aliphatic carbon atoms. In still other embodiments, the alkyl group contains 1-6 aliphatic carbon atoms. In yet other embodiments, the alkyl group contains 1-4 aliphatic carbon atoms. Examples of alkylamino include, but are not limited to, methylamino, ethylamino, iso-propylamino and the like.

[0030] Some examples of substituents of the above-described aliphatic (and other) moieties of compounds of the invention include, but are not limited to aliphatic; heteroaliphatic; aryl; heteroaryl; alkylaryl; alkylheteroaryl; alkoxy;

aryloxy; heteroalkoxy; heteroaryloxy; alkylthio; arylthio; heteroalkylthio; heteroarylthio; F; Cl; Br; I; -OH; $-NO_2$; -CN; $-CF_3$; $-CH_2CF_3$; $-CHCl_2$; $-CH_2OH$; $-CH_2CH_2OH$; $-CH_2NH_2$; $-CH_2SO_2CH_3$; $-C(O)R_x$; $-CO_2(R_x)$; $-CON(R_x)_2$; $-OC(O)R_x$; $-OCO_2R_x$; $-OCON(R_x)_2$; $-N(R_x)_2$; $-S(O)_2R_x$; $-NR_x(CO)R_x$ wherein each occurrence of R_x independently includes, but is not limited to, aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl, wherein any of the aliphatic, heteroaliphatic, alkylaryl, or alkylheteroaryl substituents described above and herein may be substituted or unsubstituted, branched or unbranched, cyclic or acyclic, and wherein any of the aryl or heteroaryl substituents described above and herein may be substituted or unsubstituted. Additional examples of generally applicable substituents are illustrated by the specific embodiments shown in the Examples that are described herein.

[0031] In general, the terms "aryl" and "heteroaryl", as used herein, refer to stable mono- or polycyclic, heterocyclic, polycyclic, and polyheterocyclic unsaturated moieties having preferably 3-14 carbon atoms, each of which may be substituted or unsubstituted. It will also be appreciated that aryl and heteroaryl moieties, as defined herein may be attached via an aliphatic, alicyclic, heteroaliphatic, heteroalicyclic, alkyl or heteroalkyl moiety and thus also include –(aliphatic)aryl, -(heteroaliphatic)aryl, -(aliphatic)heteroaryl, -(heteroaliphatic)heteroaryl, –(alkyl)aryl, -(heteroalkyl)aryl, -(heteroalkyl)aryl, and –(heteroalkyl)heteroaryl moieties. Thus, as used herein, the phrases "aryl or heteroaryl" and "aryl, heteroaryl, –(aliphatic)aryl, -(heteroaliphatic)aryl, -(aliphatic)heteroaryl, -(heteroaliphatic)heteroaryl, –(alkyl)aryl, -(heteroalkyl)aryl, -(heteroalkyl)aryl, and –(heteroalkyl)heteroaryl" are interchangeable. Substituents include, but are not limited to, any of the previously mentioned substitutents, *i.e.*, the substituents recited for aliphatic moieties, or for other moieties as disclosed herein, resulting in the formation of a stable compound. In certain embodiments of the present invention, "aryl" refers to a mono- or bicyclic carbocyclic ring system having one or two aromatic rings including, but not limited to, phenyl, naphthyl, tetrahydronaphthyl, indanyl, indenyl and the like. In certain embodiments of the present invention, the term "heteroaryl", as used herein, refers to a cyclic aromatic radical having from five to ten ring atoms of which one ring atom is selected from S,

O and N; zero, one or two ring atoms are additional heteroatoms independently selected from S, O and N; and the remaining ring atoms are carbon, the radical being joined to the rest of the molecule via any of the ring atoms, such as, for example, pyridyl, pyrazinyl, pyrimidinyl, pyrrolyl, pyrazolyl, imidazolyl, thiazolyl, oxazolyl, isooxazolyl, thiadiazolyl, oxadiazolyl, thiophenyl, furanyl, quinolinyl, isoquinolinyl, and the like.

[0032] It will be appreciated that aryl and heteroaryl groups (including bicyclic aryl groups) can be unsubstituted or substituted, wherein substitution includes replacement of one, two or three of the hydrogen atoms thereon independently with any one or more of the following moieties including, but not limited to: aliphatic; heteroaliphatic; aryl; heteroaryl; alkylaryl; alkylheteroaryl; alkoxy; aryloxy; heteroalkoxy; heteroaryloxy; alkylthio; arylthio; heteroalkylthio; heteroarylthio; F; Cl; Br; I; -OH; $-NO_2$; -CN; $-CF_3$; $-CH_2CF_3$; $-CHCl_2$; $-CH_2OH$; $-CH_2CH_2OH$; $-CH_2NH_2$; $-CH_2SO_2CH_3$; $-C(O)R_x$; $-CO_2(R_x)$; $-CON(R_x)_2$; $-OC(O)R_x$; $-OCO_2R_x$; $-OCON(R_x)_2$; $-N(R_x)_2$; $-S(O)_2R_x$; $-NR_x(CO)R_x$ wherein each occurrence of R_x independently includes, but is not limited to, aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl, wherein any of the aliphatic, heteroaliphatic, alkylaryl, or alkylheteroaryl substituents described above and herein may be substituted or unsubstituted, branched or unbranched, cyclic or acyclic, and wherein any of the aryl or heteroaryl substituents described above and herein may be substituted or unsubstituted. Additional examples of generally applicable substituents are illustrated by the specific embodiments shown in the Examples that are described herein.

[0033] The term "cycloalkyl", as used herein, refers specifically to groups having three to seven, preferably three to ten carbon atoms. Suitable cycloalkyls include, but are not limited to cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl and the like, which, as in the case of aliphatic, heteroaliphatic or heterocyclic moieties, may optionally be substituted with substituents including, but not limited to aliphatic; heteroaliphatic; aryl; heteroaryl; alkylaryl; alkylheteroaryl; alkoxy; aryloxy; heteroalkoxy; heteroaryloxy; alkylthio; arylthio; heteroalkylthio; heteroarylthio; F; Cl; Br; I; -OH; $-NO_2$; -CN; $-CF_3$; $-CH_2CF_3$; $-CHCl_2$; $-CH_2OH$; $-CH_2CH_2OH$; $-CH_2NH_2$; $-CH_2SO_2CH_3$; $-C(O)R_x$; $-CO_2(R_x)$; $-CON(R_x)_2$; $-OC(O)R_x$; -

OCO_2R_x; $-OCON(R_x)_2$; $-N(R_x)_2$; $-S(O)_2R_x$; $-NR_x(CO)R_x$ wherein each occurrence of R_x independently includes, but is not limited to, aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl, wherein any of the aliphatic, heteroaliphatic, alkylaryl, or alkylheteroaryl substituents described above and herein may be substituted or unsubstituted, branched or unbranched, cyclic or acyclic, and wherein any of the aryl or heteroaryl substituents described above and herein may be substituted or unsubstituted. Additional examples of generally applicable substituents are illustrated by the specific embodiments shown in the Examples that are described herein.

[0034] The term "heteroaliphatic", as used herein, refers to aliphatic moieties in which one or more carbon atoms in the main chain have been substituted with a heteroatom. Thus, a heteroaliphatic group refers to an aliphatic chain which contains one or more oxygen, sulfur, nitrogen, phosphorus or silicon atoms, *e.g.*, in place of carbon atoms. Heteroaliphatic moieties may be branched or linear unbranched. In certain embodiments, heteroaliphatic moieties are substituted by independent replacement of one or more of the hydrogen atoms thereon with one or more moieties including, but not limited to aliphatic; alicyclic; heteroaliphatic; heteroalicyclic; aryl; heteroaryl; alkylaryl; alkylheteroaryl; alkoxy; aryloxy; heteroalkoxy; heteroaryloxy; alkylthio; arylthio; heteroalkylthio; heteroarylthio; F; Cl; Br; I; -OH; $-NO_2$; -CN; $-CF_3$; $-CH_2CF_3$; $-CHCl_2$; $-CH_2OH$; $-CH_2CH_2OH$; $-CH_2NH_2$; $-CH_2SO_2CH_3$; $-C(O)R_x$; $-CO_2(R_x)$; $-CON(R_x)_2$; $-OC(O)R_x$; $-OCO_2R_x$; $-OCON(R_x)_2$; $-N(R_x)_2$; $-S(O)_2R_x$; $-NR_x(CO)R_x$ wherein each occurrence of R_x independently includes, but is not limited to, aliphatic, alicyclic, heteroaliphatic, heteroalicyclic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl, wherein any of the aliphatic, alicyclic, heteroaliphatic, heteroalicyclic, alkylaryl, or alkylheteroaryl substituents described above and herein may be substituted or unsubstituted, branched or unbranched, cyclic or acyclic, and wherein any of the aryl or heteroaryl substituents described above and herein may be substituted or unsubstituted. Additional examples of generally applicable substituents are illustrated by the specific embodiments shown in the Examples that are described herein.

[0035] The term "heteroalicyclic", as used herein, refers to compounds which combine the properties of heteroaliphatic and cyclic compounds and include but are

not limited to saturated and unsaturated mono- or polycyclic heterocycles such as morpholino, pyrrolidinyl, furanyl, thiofuranyl, pyrrolyl etc., which are optionally substituted with one or more functional groups, as defined herein.

[0036] Additionally, it will be appreciated that any of the alicyclic or heteroalicyclic moieties described above and herein may comprise an aryl or heteroaryl moiety fused thereto. Additional examples of generally applicable substituents are illustrated by the specific embodiments shown in the Examples that are described herein.

[0037] The terms "halo" and "halogen" as used herein refer to an atom selected from fluorine, chlorine, bromine and iodine.

[0038] The term "haloalkyl" denotes an alkyl group, as defined above, having one, two, or three halogen atoms attached thereto and is exemplified by such groups as chloromethyl, bromoethyl, trifluoromethyl, and the like.

[0039] The term "heterocycloalkyl" or "heterocycle", as used herein, refers to a non-aromatic 5-, 6- or 7- membered ring or a polycyclic group, including, but not limited to a bi- or tri-cyclic group comprising fused six-membered rings having between one and three heteroatoms independently selected from oxygen, sulfur and nitrogen, wherein (i) each 5-membered ring has 0 to 1 double bonds and each 6-membered ring has 0 to 2 double bonds, (ii) the nitrogen and sulfur heteroatoms may be optionally be oxidized, (iii) the nitrogen heteroatom may optionally be quaternized, and (iv) any of the above heterocyclic rings may be fused to an aryl or heteroaryl ring. Representative heterocycles include, but are not limited to, pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, and tetrahydrofuryl. In certain embodiments, a "substituted heterocycloalkyl or heterocycle" group is utilized and as used herein, refers to a heterocycloalkyl or heterocycle group, as defined above, substituted by the independent replacement of one, two or three of the hydrogen atoms thereon with but are not limited to aliphatic; heteroaliphatic; aryl; heteroaryl; alkylaryl; alkylheteroaryl; alkoxy; aryloxy; heteroalkoxy; heteroaryloxy; alkylthio; arylthio; heteroalkylthio; heteroarylthio; F; Cl; Br; I; - OH; $-NO_2$; -CN; $-CF_3$; $-CH_2CF_3$; $-CHCl_2$; $-CH_2OH$; $-CH_2CH_2OH$; $-CH_2NH_2$; $-CH_2SO_2CH_3$; $-C(O)R_x$; $-CO_2(R_x)$; -

$CON(R_x)_2$; $-OC(O)R_x$; $-OCO_2R_x$; $-OCON(R_x)_2$; $-N(R_x)_2$; $-S(O)_2R_x$; $-NR_x(CO)R_x$ wherein each occurrence of R_x independently includes, but is not limited to, aliphatic, heteroaliphatic, aryl, heteroaryl, alkylaryl, or alkylheteroaryl, wherein any of the aliphatic, heteroaliphatic, alkylaryl, or alkylheteroaryl substituents described above and herein may be substituted or unsubstituted, branched or unbranched, cyclic or acyclic, and wherein any of the aryl or heteroaryl substitutents described above and herein may be substituted or unsubstituted. Additional examples or generally applicable substituents are illustrated by the specific embodiments shown in the Examples, which are described herein.

[0040] As used herein, the terms "aliphatic", "heteroaliphatic", "alkyl", "alkenyl", "alkynyl", "heteroalkyl", "heteroalkenyl", "heteroalkynyl", and the like encompass substituted and unsubstituted, saturated and unsaturated, and linear and branched groups. Similarly, the terms "alicyclic", "heteroalicyclic", "heterocycloalkyl", "heterocycle" and the like encompass substituted and unsubstituted, and saturated and unsaturated groups. Additionally, the terms "cycloalkyl", "cycloalkenyl", "cycloalkynyl", "heterocycloalkyl", "heterocycloalkenyl", "heterocycloalkynyl", "aryl", "heteroaryl" and the like encompass both substituted and unsubstituted groups.

[0041] It will be appreciated that additional examples of generally applicable substitutents are illustrated by the specific embodiments shown in the Examples which are described herein, but are not limited to these Examples.

[0042] The phrase, "pharmaceutically acceptable derivative", as used herein, denotes any pharmaceutically acceptable salt, ester, or salt of such ester, of such compound, or any other adduct or derivative which, upon administration to a patient, is capable of providing (directly or indirectly) a compound as otherwise described herein, or a metabolite or residue thereof. Pharmaceutically acceptable derivatives thus include among others pro-drugs. A pro-drug is a derivative of a compound, usually with significantly reduced pharmacological activity, which contains an additional moiety, which is susceptible to removal *in vivo* yielding the parent molecule as the pharmacologically active species. An example of a pro-drug is an ester, which is cleaved *in vivo* to yield a compound of interest. Pro-drugs of a variety of compounds, and materials and methods for derivatizing the parent

compounds to create the pro-drugs, are known and may be adapted to the present invention. Certain exemplary pharmaceutical compositions and pharmaceutically acceptable derivatives will be discussed in more detail herein below.

[0043] By the term "protecting group", has used herein, it is meant that a particular functional moiety, *e.g.*, O, S, or N, is temporarily blocked so that a reaction can be carried out selectively at another reactive site in a multifunctional compound. In preferred embodiments, a protecting group reacts selectively in good yield to give a protected substrate that is stable to the projected reactions; the protecting group must be selectively removed in good yield by readily available, preferably nontoxic reagents that do not attack the other functional groups; the protecting group forms an easily separable derivative (more preferably without the generation of new stereogenic centers); and the protecting group has a minimum of additional functionality to avoid further sites of reaction. As detailed herein, oxygen, sulfur, nitrogen and carbon protecting groups may be utilized. For example, in certain embodiments, as detailed herein, certain exemplary oxygen protecting groups are utilized. These oxygen protecting groups include, but are not limited to methyl ethers, substituted methyl ethers (*e.g.*, MOM (methoxymethyl ether), MTM (methylthiomethyl ether), BOM (benzyloxymethyl ether), PMBM or MPM (p-methoxybenzyloxymethyl ether), to name a few), substituted ethyl ethers, substituted benzyl ethers, silyl ethers (*e.g.*, TMS (trimethylsilyl ether), TES (triethylsilylether), TIPS (triisopropylsilyl ether), TBDMS (t-butyldimethylsilyl ether), tribenzyl silyl ether, TBDPS (t-butyldiphenyl silyl ether), to name a few), esters (*e.g.*, formate, acetate, benzoate (Bz), trifluoroacetate, dichloroacetate, to name a few), carbonates, cyclic acetals and ketals. In certain other exemplary embodiments, nitrogen protecting groups are utilized. These nitrogen protecting groups include, but are not limited to, carbamates (including methyl, ethyl and substituted ethyl carbamates (*e.g.*, Troc), to name a few) amides, cyclic imide derivatives, N-Alkyl and N-Aryl amines, imine derivatives, and enamine derivatives, to name a few. Certain other exemplary protecting groups are detailed herein, however, it will be appreciated that the present invention is not intended to be limited to these protecting groups; rather, a variety of additional equivalent protecting groups can be readily identified using the above criteria and utilized in

the present invention. Additionally, a variety of protecting groups are described in "Protective Groups in Organic Synthesis" Third Ed. Greene, T.W. and Wuts, P.G., Eds., John Wiley & Sons, New York: 1999, the entire contents of which are hereby incorporated by reference.

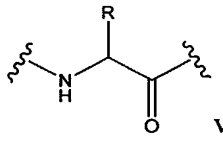
[0044] As used herein, the term "adjuvant" or "immunogenic stimulant" refers to a moiety, which, when co-administered with an immunogen, enhances the immunogenicity of the immunogen. Specifically, in certain embodiments, immunogenicity of the inventive gp120 compounds can be significantly improved if the immunizing agent(s) (*e.g.,* gp120 glycan(s) and/or construct(s) thereof) and/or composition thereof is, regardless of administration format, co-immunized with an adjuvant. Commonly, adjuvants are used as an 0.05 to 1.0 percent solution in phosphate-buffered saline. Adjuvants enhance the immunogenicity of an immunogen but are not necessarily immunogenic themselves. Adjuvants may act by retaining the immunogen locally near the site of administration to produce a depot effect facilitating a slow, sustained release of immunogen to cells of the immune system. Adjuvants can also attract cells of the immune system to an immunogen depot and stimulate such cells to elicit immune responses. As such, embodiments of this invention encompass compositions further comprising adjuvants.

[0045] Adjuvants have been used for many years to improve the host immune responses to, for example, vaccines. Intrinsic adjuvants (such as lipopolysaccharides) normally are the components of killed or attenuated bacteria used as vaccines. Extrinsic adjuvants are immunomodulators which are typically non-covalently linked to antigens and are formulated to enhance the host immune responses. Thus, adjuvants have been identified that enhance the immune response to antigens delivered parenterally. Some of these adjuvants are toxic, however, and can cause undesirable side-effects making them unsuitable for use in humans and many animals. Indeed, aluminum hydroxide and aluminum phosphate (collectively commonly referred to as alum) are routinely used as adjuvants in human and veterinary vaccines. The efficacy of alum in increasing antibody responses to diphtheria and tetanus toxoids is well established. Notwithstanding, it does have limitations. For example, alum is ineffective for influenza vaccination and inconsistently elicits a cell mediated immune response with other immunogens. The

antibodies elicited by alum-adjuvanted antigens are mainly of the IgG1 isotype in the mouse, which may not be optimal for protection by some vaccinal agents. In addition to adjuvants used for therapeutic purposes (e.g., vaccines), other adjuvants may be used for raising antibodies in animals, which antibodies may be used, for example, in diagnostic and immunoassays. Examples of such adjuvants include, but are not limited to, bacteria or liposomes. For example, suitable adjuvants include but are not limited to, saponin adjuvants (*e.g.*, GPI-0100), *Salmonella minnesota* cells, bacille Calmette-Guerin or QS21.

[0046] A wide range of extrinsic adjuvants can provoke potent immune responses to immunogens. These include saponins complexed to membrane protein antigens (immune stimulating complexes), pluronic polymers with mineral oil, killed mycobacteria and mineral oil, Freund's complete adjuvant, bacterial products such as muramyl dipeptide (MDP) and lipopolysaccharide (LPS), as well as lipid A, and liposomes.

[0047] The term "natural amino acid" as used herein refers to any one of the common, naturally occurring L-amino acids found in naturally occurring proteins: glycine (Gly), alanine (Ala), valine (Val), leucine (Leu), isoleucine (Ile), lysine (Lys), arginine (Arg), histidine (His), proline (Pro), serine (Ser), threonine (Thr), phenylalanine (Phe), tyrosine (Tyr), tryptophan (Trp), aspartic acid (Asp), glutamic acid (Glu), asparagine (Asn), glutamine (Gln), cysteine (Cys) and methionine (Met).

[0048] The term "unnatural amino acid" as used herein refers to all amino acids which are not natural amino acids. This includes, for example, α-, β-, D-, L- amino acid residues, and compounds of the general formula  wherein the side chain R is other than the amino acid side chains occurring in nature.

[0049] More generally, the term "amino acid", as used herein, encompasses natural amino acids and unnatural amino acids.

[0050] As used herein the term "biological sample" includes, without limitation, cell cultures or extracts thereof; biopsied material obtained from an animal (*e.g.*, mammal) or extracts thereof; and blood, saliva, urine, feces, semen, tears, or other body fluids or extracts thereof; or purified versions thereof. For example, the term

"biological sample" refers to any solid or fluid sample obtained from, excreted by or secreted by any living organism, including single-celled micro-organisms (such as bacteria and yeasts) and multicellular organisms (such as plants and animals, for instance a vertebrate or a mammal, and in particular a healthy or apparently healthy human subject or a human patient affected by a condition or disease to be diagnosed or investigated). The biological sample can be in any form, including a solid material such as a tissue, cells, a cell pellet, a cell extract, cell homogenates, or cell fractions; or a biopsy, or a biological fluid. The biological fluid may be obtained from any site (*e.g.* blood, saliva (or a mouth wash containing buccal cells), tears, plasma, serum, urine, bile, seminal fluid, cerebrospinal fluid, amniotic fluid, peritoneal fluid, and pleural fluid, or cells therefrom, aqueous or vitreous humor, or any bodily secretion), a transudate, an exudate (*e.g.* fluid obtained from an abscess or any other site of infection or inflammation), or fluid obtained from a joint (*e.g.* a normal joint or a joint affected by disease such as rheumatoid arthritis, osteoarthritis, gout or septic arthritis). The biological sample can be obtained from any organ or tissue (including a biopsy or autopsy specimen) or may comprise cells (whether primary cells or cultured cells) or medium conditioned by any cell, tissue or organ. In certain embodiments, the biological sample is obtained from the prostate epithelium. Biological samples may also include sections of tissues such as frozen sections taken for histological purposes. Biological samples also include mixtures of biological molecules including proteins, lipids, carbohydrates and nucleic acids generated by partial or complete fractionation of cell or tissue homogenates. Although the sample is preferably taken from a human subject, biological samples may be from any animal, plant, bacteria, virus, yeast, etc. The term *animal*, as used herein, refers to humans as well as non-human animals, at any stage of development, including, for example, mammals, birds, reptiles, amphibians, fish, worms and single cells. Cell cultures and live tissue samples are considered to be pluralities of animals. In certain exemplary embodiments, the non-human animal is a mammal (*e.g.*, a rodent, a mouse, a rat, a rabbit, a monkey, a dog, a cat, a sheep, cattle, a primate, or a pig). An animal may be a transgenic animal or a human clone. If desired, the biological sample may be subjected to preliminary processing, including preliminary separation techniques. In certain emboidments, the biological sample is

taken from a male human subject. In certain exemplary embodiment, the biological sample has been processed so that the gp120 glycan concentration out of the total glycan concentration in the original sample is increased. In certain exemplary embodiments, the sample may be purified serum gp120, purified gp120 glycoprotein, purified gp120 glycoprotein that has undergone sialidase digestion, purified gp120 glycans obtained from deglycosylated gp120 glycoprotein, or any combination thereof. It will be appreciated that the term "biological sample", as used herein, encompasses any combination of gp120 materials obtained from any biological sources (*e.g.*, as detailed above) or by any processes that may be used to obtain gp120 glycan from the original sample (*e.g.*, extraction, purification, glycoprotein deglycosylation, sialidase digestion, etc.).

[0051] As used herein, the term "isolated" when applied to the compounds of the present invention, refers to such compounds that are (i) separated from at least some components with which they are associated in nature or when they are made and/or (ii) produced, prepared or manufactured by the hand of man. In certain embodiments, isolated compounds of the invention are not substantially contaminated with, or otherwise in contact with any other compound. Accordingly, the present invention provides compounds of formula **(I)** and/or **(II)** in substantially pure form, *i.e.*, in a purity of greater than about 95% by weight (not including H_2O or salt content, which is to be expected, for example, from lyophilized peptides and glycopeptides), preferably greater than about 98%, and more preferably greater than about 99% by weight. In one aspect, the impurity in contact with a compound of formula **(I)** and/or **(II)** of the invention is an organic chemical, *e.g.*, an organic solvent. In another aspect, the impurity in contact with a compound of formula **(I)** and/or **(II)** is another compound of formula **(I)** and/or **(II)**. Thus, in one aspect, the present invention provides a compound of formula **(I)** and/or **(II)** that is pure in that it is not in contact with another compound of formula **(I)** and/or **(II)**.

[0052] As used herein, the term "glycoconjugate" refers to one or more glycans covalently linked to a peptidic or non-peptidic backbone.

[0053] As used herein, the term "gp120 glycan" refers to a carbohydrate domain present on gp120. More specifically, gp120 glycan designates the carbohydrate portion of compounds of formula **(I)**, **(II)** and/or **(III)** described herein. In certain

embodiments, the term refers to compounds of formula **(I), (II)** and/or **(III)** where R^4 is a moiety other than a peptide, protein or other polymeric construct.

[0054] As used herein, the term "gp120 glycopeptide" refers to compounds of formula **(I), (II)** and/or **(III)** where R^4 comprises a peptide moiety covalently linked to the rest of the construct either directly (*e.g.*, through N or O) or through a crosslinker.

[0055] As used herein, the term "eliciting an immune response" is defined as initiating, triggering, causing, enhancing, improving or augmenting any response of the immune system, for example, of either a humoral or cellular nature. The initiation or enhancement of an immune response can be assessed using assays known to those skilled in the art including, but not limited to, antibody assays (for example ELISA assays). In certain exemplary embodiments, the inventive gp120 glycans and/or glycoconjugates thereof, and the methods of the present invention essentially trigger or enhance primarily a humoral immune response.

BRIEF DESCRIPTION OF THE DRAWING

[0056] Figure 1 depicts structures of gp120 glycopeptides **1-2**.

DETAILED DESCRIPTION OF CERTAIN PREFERRED EMBODIMENTS OF THE INVENTION

[0057] As discussed above, the desire to develop improved methods for the preparation of synthetic vaccines has led to increased research efforts directed toward the synthesis of naturally occurring complex carbohydrate antigens, as well as novel complex structures (*e.g.*, glycopeptides or other glycoconjugates) incorporating these antigenic structures. As is often the case during the course of any such large synthetic undertaking, improved synthetic methods are often developed that can be applied universally. In particular, synthetic studies of naturally occurring antigenic structures has led to the development of novel methodologies enabling the development of heretofore unavailable synthetic carbohydrate-based vaccines. For a review, see Danishefsky, S.J.; Allen, J.R., *Angew. Chem. Int. Ed. Engl.* **2000**, *39*, 836-863, and references cited therein.

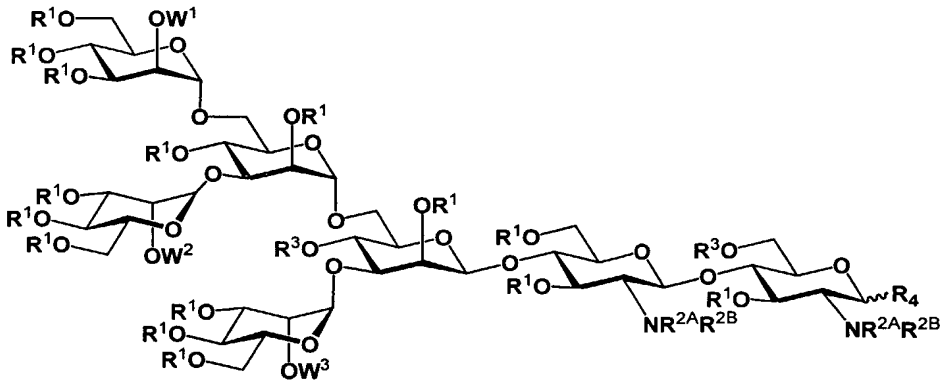
[0058] Significantly, the present invention provides novel methodologies for the synthesis of complex carbohydrates and related therapeutic compounds (*e.g.*, glycans and/or glycoconjugates thereof). In particular, in the context of synthetic studies developed for the total synthesis of glycosylated fragments of gp120 and conjugates thereof, generalized methodologies were developed for the improved synthesis of complex carbohydrate structures. This general synthetic method encompasses the realization that the incorporation of an amino group at the reducing end of a carbohydrate acceptor allows for accessibility to complex N-linked carbohydrate conjugates. In yet another aspect, the present invention also provides the recognition that for certain protected carbohydrates, the amino carbohydrate moieties can serve as useful precursors that can be utilized ultimately for the synthesis of complex N-linked glycopeptides or other glycoconjugates.

[0059] Specific examples, particularly with respect to the total synthesis of N-acetyllactosamine-type glycans and their incorporation into gp120 glycopeptide fragments and other non-peptidic glycoconjugates are described in more detail below, along with certain general methodologies developed during the course of these syntheses. It will be appreciated by one of ordinary skill in the art that these examples are not intended to be limiting; rather all equivalents are intended to be incorporated into the scope of the present invention.

[0060] **1) Inventive Compounds**

[0061] As mentioned above, the total synthesis of complex antigenic structures has led to significant development in methodologies for complex carbohydrate synthesis. Of particular recent interest is the naturally occurring antigenic gp120 glycans; e.g., "high-mannose"- and "hybrid"-type glycoforms thereof (See constructs **1-2** in Figure 1) which heretofore had not yet been synthesized.

[0062] Thus, in one aspect of the present invention, the synthesis of the complex gp120 carbohydrate domains has been achieved and an isolated compound of formula **(I)** having the structure as shown below is provided:

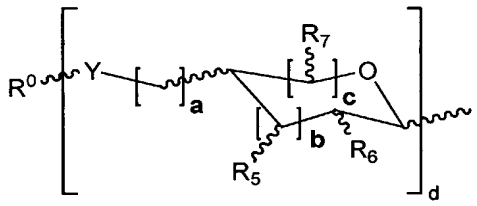


(I)

wherein each occurrence of R^1 is independently hydrogen or an oxygen protecting group;

each occurrence of R^{2A} and R^{2B} is independently hydrogen or a nitrogen protecting group;

each occurrence of R^3 is independently hydrogen, a protecting group or a carbohydrate domain comprising a saccharide moiety having the structure:



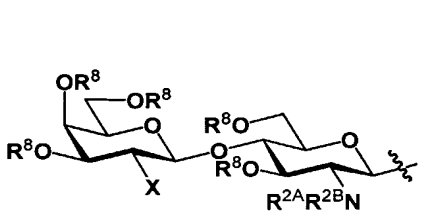
wherein Y is NH or O; wherein a, b and c are each independently 0, 1 or 2; d is an integer from 1-3; with the proviso that the d bracketed structure represents a furanose or pyranose moiety and the sum of b and c is 1 or 2; wherein R^0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein each occurrence of R^5, R^6 and R^7 is independently hydrogen, OH, OR^i, $NR^{ii}R^{iii}$, $NHCOR^i$, F, CH_2OH, CH_2OR^i, or a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of R^i, R^{ii} and R^{iii} is independently hydrogen, a protecting group, a sialic acid moiety, CHO, $COOR^{iv}$, or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group, or R^{ii} and R^{iii}, taken together with the nitrogen atom to which they are attached, form a substituted or unsubstituted heterocyclic or heteroaryl moiety; and wherein each

occurrence of R^{iv} is independently H, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group;

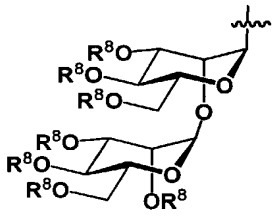
W^1, W^2 and W^3 are independently optionally substituted mannose, galactose or lactosamine moieties;

and wherein R^4 is $-OR^{4A}$ or $-NHR^{4A}$; wherein R^{4A} is hydrogen, aliphatic, heteroaliphatic, aryl, heteroaryl, an amino acyl moiety, an amino acyl residue of a peptide, an amino acyl residue of a protein, or R^{4A} comprises a protein, peptide or lipid moiety covalently linked to the rest of the construct, or to the N or O atom to which it is attached, either directly or through a crosslinker.

[0063] In certain embodiments, W^3 is R^1, R^3, as defined above, or a moiety having the structure:

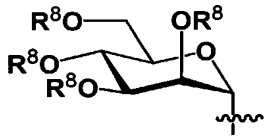


or



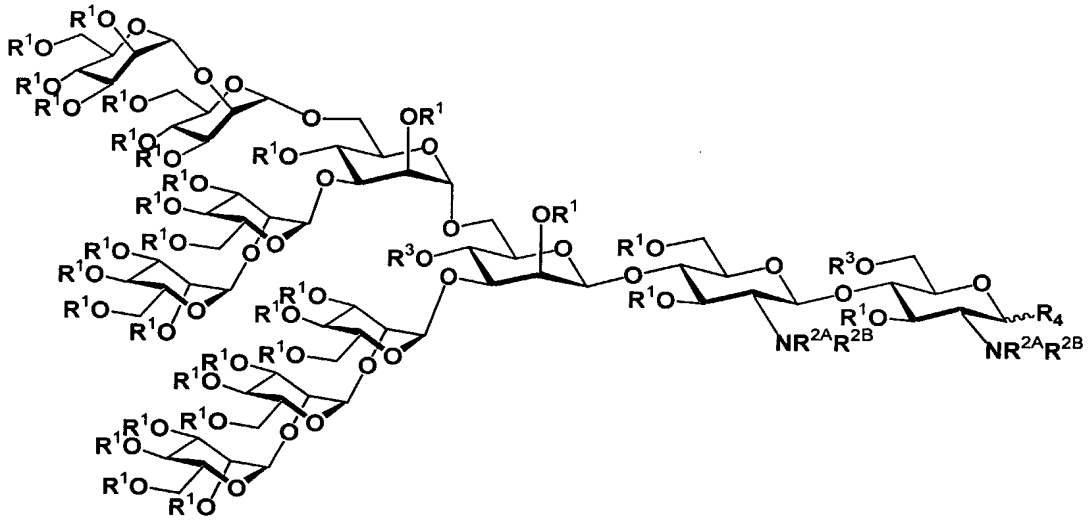
wherein X is $-OR^1$ or $-NR^{2A}R^{2B}$; and each occurrence of R^8 is independently R^1 or a sialic acid moiety.

[0064] In certain embodiments, W^1 and W^2 are independently R^1, R^3 or a moiety having the structure:



wherein each occurrence of R^8 is independently R^1 or a sialic acid moiety.

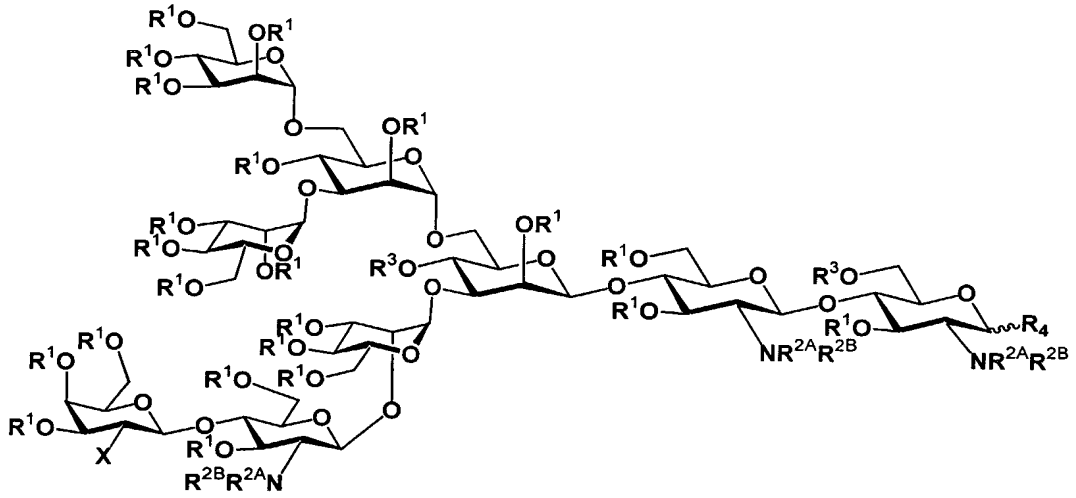
[0065] In certain embodiments, a compound of formula **(II)** having the structure as shown below is provided:



(II)

wherein R^1, R^{2A}, R^{2B}, R^3 and R^4 are as defined above.

[0066] In certain embodiments, a compound of formula **(III)** having the structure as shown below is provided:

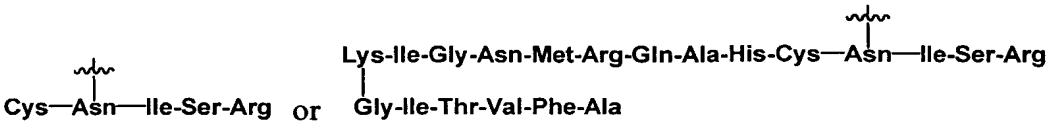


(III)

wherein R^1, R^{2A}, R^{2B}, R^3 and R^4 are as defined above and X is OR^1 or $NR^{2A}R^{2B}$.

[0067] In certain embodiments, compounds of formula **(I)**, **(II)** or **(III)** exclude naturally occurring gp120 (e.g., a glycan domain found on naturally occurring gp120 glycoprotein).

[0068] In certain embodiments, when R^4 comprises a peptide, the peptide is either identical to or closely related to that of gp120 near an N-glycosylation site. In certain exemplary embodiments, the peptide has the structure:



or truncated, elongated or derivatized version thereof; wherein any one or more of the amino acid residues may bear one or more protecting groups. For the purpose of the invention, "truncated", refers to a peptide fragment comprising no fewer than about 6 amino acid residues; "elongated", refers to a peptide comprising no more than about 60 amino acid residues; and "derivatized" refers to a peptide in which at least one, but not more than about 2 out of every 10, amino acid residues have been added and/or deleted; and/or in which at least one amino acid residue has been substituted with a natural or non-natural amino acid residue so that the resulting peptide has a sequence identity equal or greater to about 70% with the original peptide.

[0069] In certain exemplary embodiments, for compounds of formula **(I)**, **(II)** and **(III)** above, each occurrence of R^1 is independently an oxygen protecting group. In certain other exemplary embodiments, each occurrence of R^1 is independently hydrogen, alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, aryl, heteroaryl, alkylaryl, alkylheteroaryl, $-Si(R^{1A})_3$, $-C(=O)R^{1A}$, $-C(=S)R^{1A}$, $-C(=NR^{1A})R^{1B}$, $-SO_2R^{1A}$, wherein R^{1A} and R^{1B} are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl, heteroaliphatic, heteroalicyclic, aryl, heteroaryl, $-C(=O)R^{1C}$ or $-ZR^{1C}$, wherein Z is –O-, -S-, $-NR^{1D}$, wherein each occurrence of R^{1C} and R^{1D} is independently hydrogen, or an alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl, heteroaliphatic, heteroalicyclic, aryl or heteroaryl moiety. In yet other exemplary embodiments, each occurrence of R^1 is independently hydrogen, alkylaryl, $-Si(R^{1A})_3$ or $-C(=O)R^{1A}$, wherein R^{1A} is as defined above. In yet other exemplary embodiments, each occurrence of R^1 is

independently hydrogen, Bn or Bz. In certain other exemplary embodiments, each occurrence of R^1 is independently hydrogen.

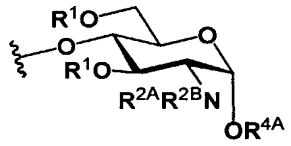
[0070] In certain other exemplary embodiments, for compounds of formula **(I)**, **(II)** and **(III)** above, for each occurrence of $-NR^{2A}R^{2B}$, at least one occurrence of R^{2A} or R^{2B} is independently a nitrogen protecting group. In certain other exemplary embodiments, each occurrence of $-NR^{2A}R^{2B}$, R^{2A} and R^{2B} is independently hydrogen, alkyl, alkenyl, $-C(=O)R^{2C}$, $-C(=O)OR^{2C}$, $-SR^{2C}$, SO_2R^{2C}, or R^{2A} and R^{2B}, taken together with the nitrogen atom to which they are attached, form a substituted or unsubstituted heterocyclic or heteroaryl moiety; wherein each occurrence of R^{2C} is independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl, heteroaliphatic, heteroalicyclic, aryl, heteroaryl, $-C(=O)R^{2D}$ or $-ZR^{2D}$, wherein Z is –O-, -S-, $-NR^{2E}$, wherein each occurrence of R^{2D} and R^{2E} is independently hydrogen, or an alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl, heteroaliphatic, heteroalicyclic, aryl or heteroaryl moiety. In certain exemplary embodiments, for each occurrence of $-NR^{2A}R^{2B}$, at least one occurrence of R^{2A} or R^{2B} is independently $-C(=O)R^{2A}$ or SO_2R^{2A}; or R^{2A} and R^{2B}, taken together with the nitrogen atom to which they are attached, form a substituted or unsubstituted heterocyclic or heteroaryl moiety. In yet other exemplary embodiments, for each occurrence of $-NR^{2A}R^{2B}$, at least one occurrence of R^{2A} or R^{2B} is independently $-C(=O)R^{2C}$ or SO_2R^{2C} wherein R^{2C} is as defined above, or R^{2A} and R^{2B}, taken together with the nitrogen atom to which they are attached, form an azide or a substituted or unsubstituted phthalimide moiety. In yet other exemplary embodiments, for each occurrence of $-NR^{2A}R^{2B}$, at least one occurrence of R^{2A} or R^{2B} is independently acyl, $-SO_2Ph$ or R^{2A} and R^{2B}, taken together with the nitrogen atom to which they are attached, form an azide or a substituted or unsubstituted phthalimide moiety. In certain other exemplary embodiments, each occurrence of $-NR^{2A}R^{2B}$ is –NHAc.

[0071] In certain other embodiments, for compounds of formula **(III)** above, X is $-OR^1$, wherein R^1 is as defined generally above and in classes and subclasses herein.

[0072] In certain other embodiments, for compounds of formula **(I)**, **(II)** and **(III)** above, each occurrence of R^3 is independently R^1, wherein R^1 is as defined generally above and in classes and subclasses herein. In certain embodiments, each occurrence of R^3 is independently hydrogen, alkylaryl, $-Si(R^{3A})_3$ or $-C(=O)R^{3A}$, wherein R^{3A} is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl, heteroaliphatic, heteroalicyclic, aryl, heteroaryl, $-C(=O)R^{3B}$ or $-ZR^{3B}$, wherein Z is –O-, -S-, $-NR^{3C}$, wherein each occurrence of R^{3B} and R^{3C} is independently hydrogen, or an alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl, heteroaliphatic, heteroalicyclic, aryl or heteroaryl moiety. In yet other exemplary embodiments, each occurrence of R^3 is independently hydrogen, Bn or Bz. In certain other exemplary embodiments, each occurrence of R^3 is independently hydrogen.

[0073] In certain other embodiments, for compounds of formula **(I)**, **(II)** and **(III)** above, each occurrence of R^1 and R^3 is independently hydrogen, alkylaryl, $-Si(R^{3A})_3$ or $-C(=O)R^{3A}$, wherein R^{3A} is as defined above. In yet other exemplary embodiments, each occurrence of R^1 and R^3 is independently hydrogen, Bn or Bz. In certain other exemplary embodiments, each occurrence of R^1 is Bn and each occurrence of R^3 is Bz. In certain other exemplary embodiments, each occurrence of R^1 and R^3 is independently hydrogen.

[0074] In certain embodiments, for compounds of formula **(I)**, **(II)** and **(III)** above, R^4 is $-OR^{4A}$ and the saccharide unit bearing R^4 has the structure:



wherein R^1, R^{2A} and R^{2B} are as defined generally above and in classes and subclasses herein; R^{4A} is hydrogen, alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, aryl, heteroaryl, alkylaryl, alkylheteroaryl, an amino acyl moiety, an amino acyl residue of a peptide, an amino acyl residue of a protein, $-Si(R^{4B})_3$, $-C(=O)R^{4B}$, $-C(=S)R^{4B}$, $-C(=NR^{4B})R^{4C}$, $-SO_2R^{4B}$, wherein R^{4B} and R^{4C} are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl,

cycloalkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl, heteroaliphatic, heteroalicyclic, aryl, heteroaryl, -C(=O)R^{4D} or –ZR^{4D}, wherein Z is –O-, -S-, -NR^{4E}, wherein each occurrence of R^{4D} and R^{4E} is independently hydrogen, or an alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl, heteroaliphatic, heteroalicyclic, aryl or heteroaryl moiety; or R^{4A} comprises a protein, peptide or lipid moiety covalently linked to the O atom to which it is attached, either directly or through a crosslinker. In yet other exemplary embodiments, R^{4A} is -$Si(R^{4B})_3$, wherein R^{4B} is as defined above. In yet other exemplary embodiments, R^{4A} is TBS. In yet other exemplary embodiments R^{4A} comprises a serine (ser) amino acyl residue. In yet other exemplary embodiments R^{4A} comprises a threonine (Thr) amino acyl residue. In yet other exemplary embodiments R^{4A} comprises a peptide attached to O through a serine (Ser) residue. In yet other exemplary embodiments R^{4A} comprises a peptide attached to O through a Threonine (Thr) residue.

[0075] In certain embodiments, for compounds of formula **(I)**, **(II)** and **(III)** above, R^4 is –NHR^{4A} and the saccharide unit bearing R^4 has the structure:



wherein R^1, R^{2A} and R^{2B} are as defined generally above and in classes and subclasses herein; and R^{4A} is hydrogen, aliphatic, heteroaliphatic, aryl, heteroaryl, an amino acyl moiety, an amino acyl residue of a peptide, an amino acyl residue of a protein, or R^{4A} comprises a protein, peptide or lipid moiety covalently linked to the rest of the construct, or to the N atom to which it is attached, either directly or through a crosslinker.

[0076] In certain exemplary embodiments, R^{4A} is hydrogen.

[0077] In certain other exemplary embodiments, R^{4A} comprises an amino acyl residue of a peptide whose structure is either identical or closely related to that of gp120 near an N-glycosylation site.

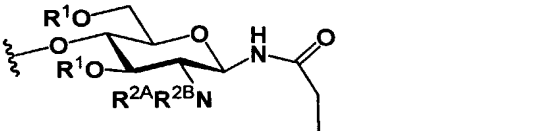
[0078] In certain other exemplary embodiments, R^{4A} comprises an Asparagine residue (Asn) of a peptide whose structure is either identical or closely related to that of gp120 near an N-glycosylation site.

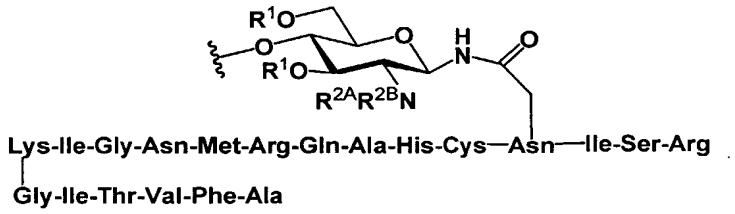
[0079] For the purpose of the invention, a peptide whose structure is "closely related to that of gp120 near an N-glycosylation site" designates a gp120 peptide fragment, or truncated, elongated or derivatized version thereof, comprising ≤ about 60 amino acid residues, wherein one amino acid residue bears an N-glycosylation site, at least one amino acid residue has been added, deleted and/or substituted with a natural or non-natural amino acid residue, so that the resulting peptide has a sequence identity greater or equal to about 70% with the original gp120 peptide fragment. In certain embodiments, the peptide comprises ≤ about 55 amino acid residues. In certain embodiments, the peptide comprises ≤ about 50 amino acid residues. In certain embodiments, the peptide comprises ≤ about 45 amino acid residues. In certain embodiments, the peptide comprises ≤ about 40 amino acid residues. In certain embodiments, the peptide comprises ≤ about 35 amino acid residues. In certain embodiments, the peptide comprises ≤ about 30 amino acid residues. In certain embodiments, the peptide comprises ≤ about 25 amino acid residues. In certain embodiments, the peptide comprises ≤ about 20 amino acid residues. In certain embodiments, the peptide has a sequence identity greater or equal to about 75% with the original gp120 peptide fragment. In certain other embodiments, the peptide has a sequence identity greater or equal to about 80% with the original gp120 peptide fragment. In certain other embodiments, the peptide has a sequence identity greater or equal to about 85% with the original gp120 peptide fragment. In certain other embodiments, the peptide has a sequence identity greater or equal to about 90% with the original gp120 peptide fragment. In certain other embodiments, the peptide has a sequence identity greater or equal to about 95% with the original gp120 peptide fragment.

[0080] A peptide whose structure is "identical to that of gp120 near an N-glycosylation site" designates a gp120 peptide fragment of a naturally occurring gp120 glycoprotein, comprising ≤ about 60 amino acid residues, wherein one amino acid residue bears an N-glycosylation site. In certain embodiments, the peptide comprises ≤ about 55 amino acid residues. In certain embodiments, the peptide comprises ≤ about 50 amino acid residues. In certain embodiments, the peptide comprises ≤ about 45 amino acid residues. In certain embodiments, the peptide comprises ≤ about 40 amino acid residues. In certain embodiments, the peptide

comprises ≤ about 35 amino acid residues. In certain embodiments, the peptide comprises ≤ about 30 amino acid residues. In certain embodiments, the peptide comprises ≤ about 25 amino acid residues. In certain embodiments, the peptide comprises ≤ about 20 amino acid residues.

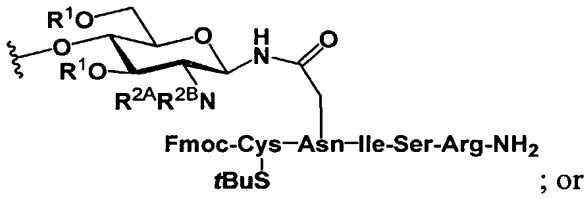
[0081] In certain embodiments, for compounds of formula **(I)**, **(II)** and **(III)** above, R^4 is $-NHR^{4A}$ wherein R^{4A} comprises an Asparagine residue (Asn) of a peptide whose structure is either identical or closely related to that of gp120 near an N-glycosylation site and the saccharide unit bearing R^4 has the structure:

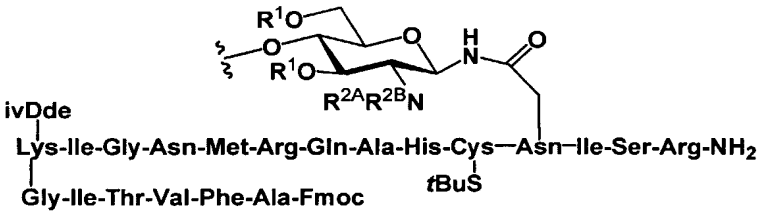




wherein R^1, R^{2A} and R^{2B} are as defined generally above and in classes and subclasses herein and wherein any of the amino acid residues may bear one or more protecting groups.

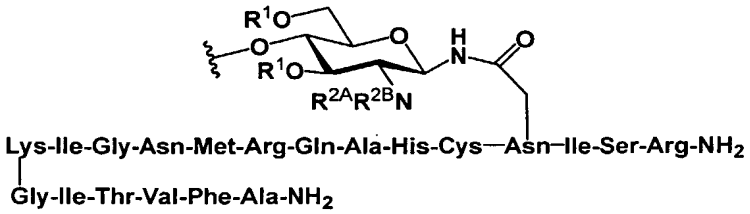
[0082] In certain exemplary embodiments, the saccharide unit bearing R^4 has the structure:





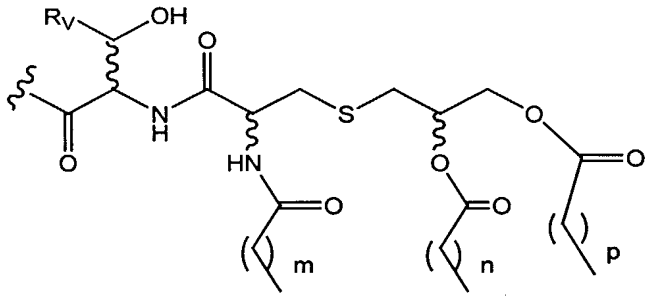
wherein R^1, R^{2A} and R^{2B} are as defined generally above and in classes and subclasses herein.

[0083] In certain other exemplary embodiments, the saccharide unit bearing R^4 has the structure:



wherein R^1, R^{2A} and R^{2B} are as defined generally above and in classes and subclasses herein.

[0084] In certain embodiments, any of the isolated compounds, glycopeptides and/or glycoconjugates described herein may be further conjugated to an immunogenic carrier. In certain exemplary embodiments, the carrier is a protein, a peptide or a lipid. In certain other exemplary embodiments, the carrier is Bovine Serum Albumin (BSA), Keyhole Limpet Hemocyanin (KLH) or polylysine. In certain other embodiments, the carrier is is a lipid carrier having the structure:

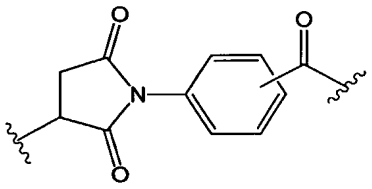


wherein m, n and p are each independently integers between about 8 and 20; and R_V is hydrogen, substituted or unsubstituted linear or branched chain lower alkyl or substituted or unsubstituted phenyl. In certain exemplary embodiments, m′, n′ and p′ are each 14 and the lipid is tripalmitoyl-S-glycerylcysteinylserine (*e.g.*, PamCys).

[0085] It will be appreciated that the carrier can be linked to the rest of the construct either directly or through a crosslinker, and thus R^4 encompasses proteins, peptides, and lipids, as well as (crosslinker-protein), (crosslinker-peptide) and (crosslinker-lipid) moieties.

[0086] Crosslinkers suited to the invention are widely known in the art (see, for example, 1994 Pierce Technical Handbook: cross-linking (Appendix A), which is available at http://www.piercenet.com/ resources/browse.cfm?fldID=184), including

bromoacetic NHS ester, 6-(iodoacetamido)caproic acid NHS ester, maleimidoacetic acid NHS ester, maleimidobenzoic acide NHS ester, etc. In certain preferred embodiments, the crosslinker is MMCCH (4-(maleimidomethyl) cyclohexane-1-carboxyl hydrazide). In certain other preferred embodiments, the crosslinker is MBS (m-maleimidobenzoyl acid N-Hydroxysuccinimidyl ester). In certain embodiments, the crosslinker is a fragment having the structure:



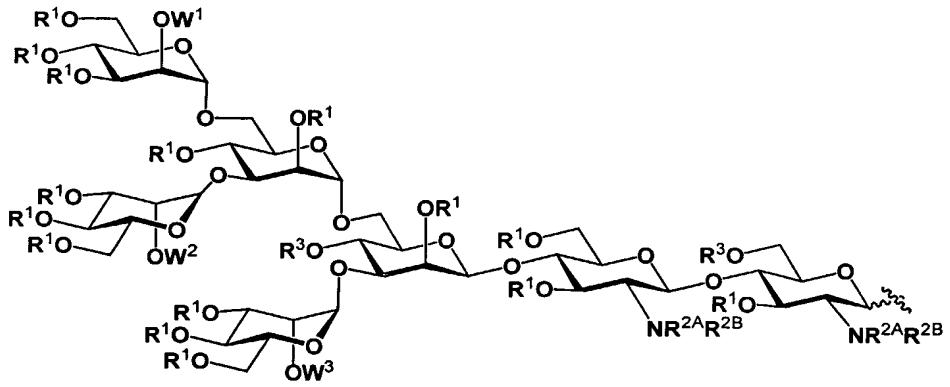
;

whereby said structure is generated upon conjugation of a maleimidobenzoic acid N-hydroxy succinimide ester with a suitable functionality on R^4.

[0087] Recently, crystallographic studies revealed that the broadly neutralizing human antibody 2G12, which binds with nanomolar affinity to gp120, contains multiple, distinct binding sites for carbohydrates (e.g., glycans expressed on gp120) [see, Calarese *et al.*, "Antibody domain exchange is an immunological solution to carbohydrate cluster recognition", *Science*, **300**:2065-2071, 2003; which is incorporated herein by reference in its entirety]. It was proposed that these multiple binding sites of 2G12 were important for high-affinity interaction of the antibody with the dense array of oligomannose sugars on the surface of gp120.

[0088] Therefore, without wishing to be bound to any particular theory, Applicant proposes that constructs comprising several carbohydrate domains present on the surface of gp120, or analogs or derivatives thereof, could therefore elicit a humoral immune response comprising antibodies with enhanced binding affinity for gp120, and therefore have greater potential in the development of HIV vaccines.

[0089] Thus, in certain embodiments, there is provided an antigenic construct comprising one or more carbohydrate domains having the structure:

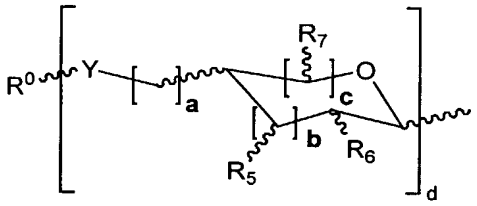


(I^{det})

wherein each occurrence of R^1 is independently hydrogen or an oxygen protecting group;

each occurrence of R^{2A} and R^{2B} is independently hydrogen or a nitrogen protecting group;

each occurrence of R^3 is independently hydrogen, a protecting group or a carbohydrate domain comprising a saccharide moiety having the structure:



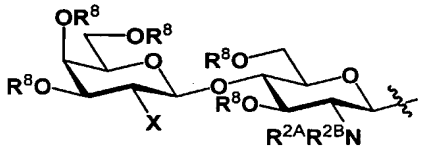
wherein Y is NH or O; wherein a, b and c are each independently 0, 1 or 2; d is an integer from 1-3; with the proviso that the d bracketed structure represents a furanose or pyranose moiety and the sum of b and c is 1 or 2; wherein R^0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein each occurrence of R^5, R^6 and R^7 is independently hydrogen, OH, OR^i, $NR^{ii}R^{iii}$, $NHCOR^i$, F, CH_2OH, CH_2OR^i, or a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of R^i, R^{ii} and R^{iii} is independently hydrogen, a protecting group, a sialic acid moiety, CHO, $COOR^{iv}$, or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group, or R^{ii} and R^{iii}, taken together with the nitrogen atom to which they are attached, form a substituted or unsubstituted heterocyclic or heteroaryl moiety; and wherein each

occurrence of R^{iv} is independently H, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group;

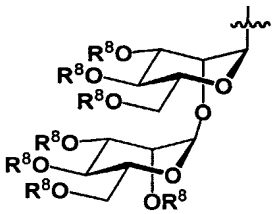
W^1, W^2 and W^3 are independently optionally substituted mannose, galactose or lactosamine moieties;

wherein each carbohydrate domain is independently covalently bound to a linker system, said linker system being a peptide or non-peptide nature, and wherein the linker system may be cyclic or acyclic.

[0090] In certain embodiments, W^3 is R^1, R^3, as defined above, or a moiety having the structure:

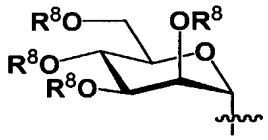


or



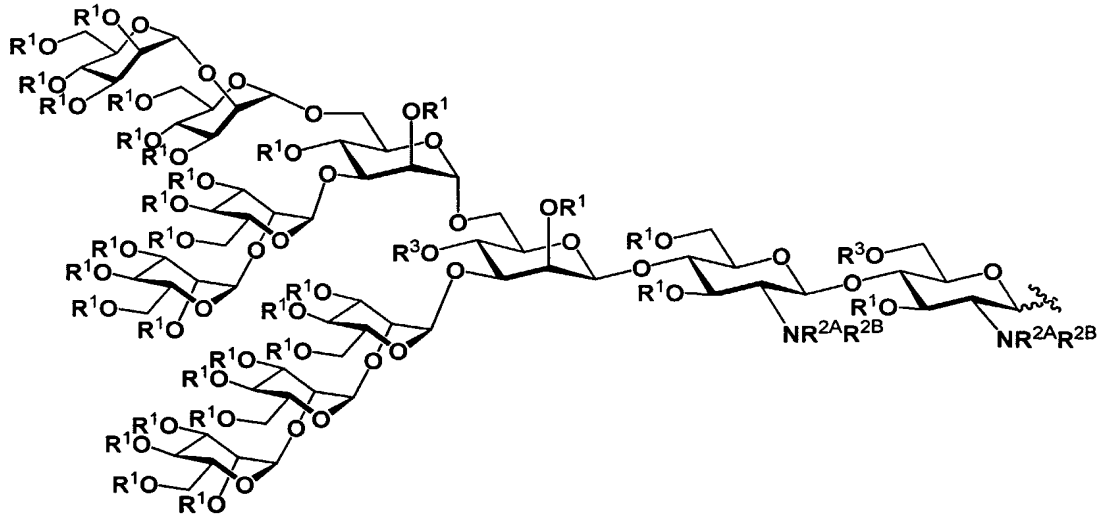
wherein X is $-OR^1$ or $-NR^{2A}R^{2B}$; and each occurrence of R^8 is independently R^1 or a sialic acid moiety.

[0091] In certain other embodiments, W^1 and W^2 are independently R^1, R^3 or a moiety having the structure:



wherein each occurrence of R^8 is independently R^1 or a sialic acid moiety.

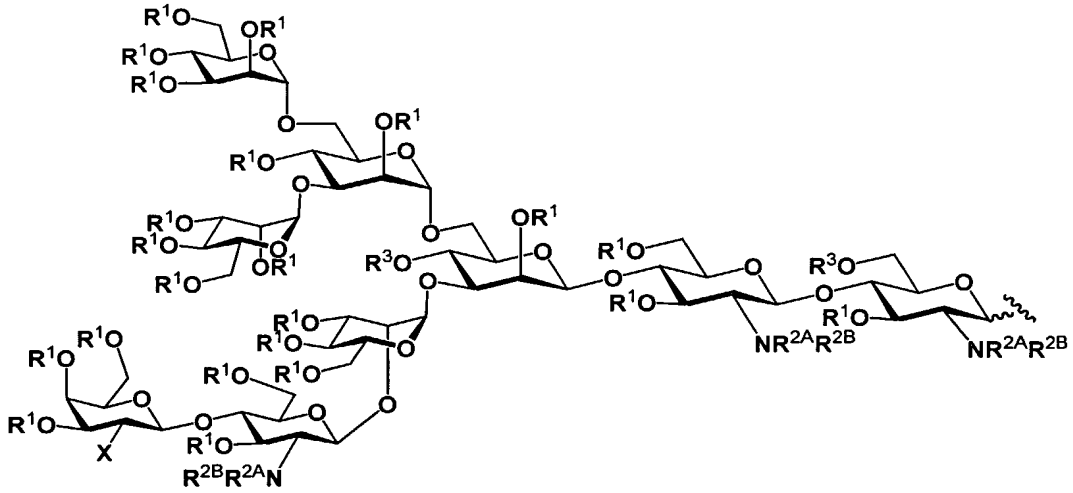
[0092] In yet other embodiments, inventive constructs comprise one or more carbohydrate domains having the structure:



(II^{det})

wherein R^1, R^3, R^{2A} and R^{2B} are as defined above for **(I^{det})**.

[0093] In yet other embodiments, inventive constructs comprise one or more carbohydrate domains having the structure:



(III^{det})

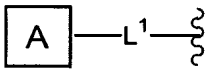
wherein R^1, R^3, R^{2A} and R^{2B} are as defined above for **(I^{det})**.

[0094] In certain embodiments, some or all of carbohydrate domains are O-linked to the linker system. In certain other embodiments, some or all of carbohydrate domains are N-linked to the linker system. In yet other embodiments, the linker system is a peptide. In certain embodiments, the linker system is a cyclic peptide. In certain other embodiments, the linker system is cyclodextrin. In certain embodiments, the linker system is a peptide and comprises two or more

carbohydrate domains covalently attached thereto, wherein the peptide sequence between each point of attachment of the carbohydrate domains comprises a cysteine residue. In certain embodiments, the multi-glycan construct is prepared by Native Chemical Ligation. In certain embodiments, the inventive constructs are symmetrical, nonsymmetrical and mixed (N-linked and O-linked carbohydrates). In certain embodiments, the linker system is designed to approximate the spatial position(s) of carbohydrate(s) in gp120. In yet other embodiments, the linker system is further attached to a carrier immunostimulant.

[0095] In certain embodiments, inventive constructs comprising one or more carbohydrate domains of the formula **(I^{det})**, **(II^{det})** or **(III^{det})** are similar to multi-antigenic constructs described in U.S.S.N. 09/083,776 filed March 25, 1998, 09/276,595 filed March 25, 1999, 10/600,012 filed June 19, 2003, 09/641,742 filed August 18, 2000, 10/ 209,618 filed July 31, 2002 and 10/430,822, filed December 3, 2003 and entitled "Clustered Multi-Antigenic Carbohydrate Constructs, Methods for their Preparation, and Uses Thereof"; each of the above applications is hereby incorporated by reference in its entirety. Guidance for preparing such constructs can be found, *inter alia*, in the above-cited applications.

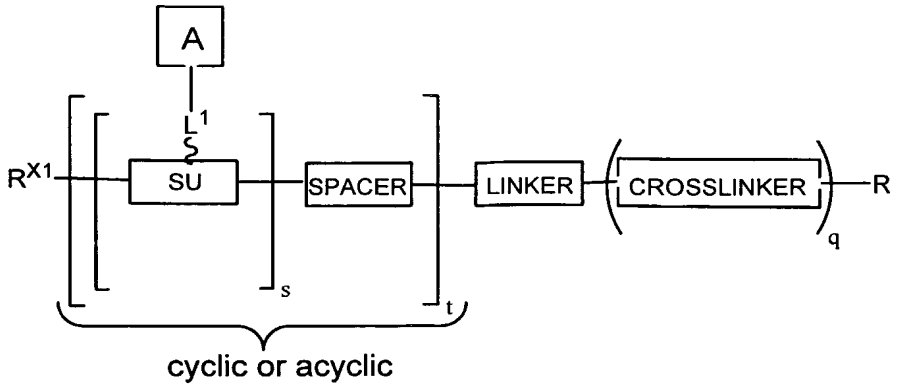
[0096] For example, the present invention encompasses clustered glycoconjugates comprising a cyclic or acyclic backbone made up of two or more amino acids or other structural units, wherein one or more of said amino acids or structural units is/are independently substituted with a glycosidic moiety having the structure:



wherein each occurrence of L^1 is independently a substituted or unsubstituted, linear or branched, cyclic or acyclic, saturated or unsaturated aliphatic or heteroaliphatic moiety; and

each occurrence of A is independently a carbohydrate domain of formula **(I^{det})**, **(II^{det})** or **(III^{det})**.

[0097] In certain embodiments, the invention encompasses clustered multi-antigenic constructs having the structure:



wherein q is 0 or 1;

each occurrence of s is independently an integer from 2-20;

t is an integer from 1-6;

R^{X1} is hydrogen, alkyl, acyl, aryl, heteroaryl, -alkyl(aryl), -alkyl(heteroaryl) or a nitrogen protecting group; or R^{X1} is covalently bound to a substituent on the last occurrence of the spacer, thereby forming a cyclic backbone;

R is hydrogen or an immunogenic carrier;

each occurrence of the structural unit SU is independently a substituted or unsubstituted aliphatic, heteroaliphatic, aryl, heteroaryl or peptidic moiety;

each occurrence of the spacer is independently a substituted or unsubstituted aliphatic, heteroaliphatic, aryl, heteroaryl or peptidic moiety;

the linker is either a free carboxylic acid, –O-, (carboxamido)alkyl carboxamide, MBS, primary carboxamide, mono- or dialkyl carboxamide, mono- or diarylcarboxamide, linear or branched chain (carboxy)alkyl carboxamide, linear or branched chain (alkoxycarbonyl)alkyl-carboxamide, linear or branched chain (carboxy)arylalkylcarboxamide, linear or branched chain (alkoxycarbonyl)alkylcarboxamide, an oligoester fragment comprising from 2 to about 20 hydroxy acyl residues, a peptidic fragment comprising from 2 to about 20 amino acyl residues, or a linear or branched chain alkyl or aryl carboxylic ester;

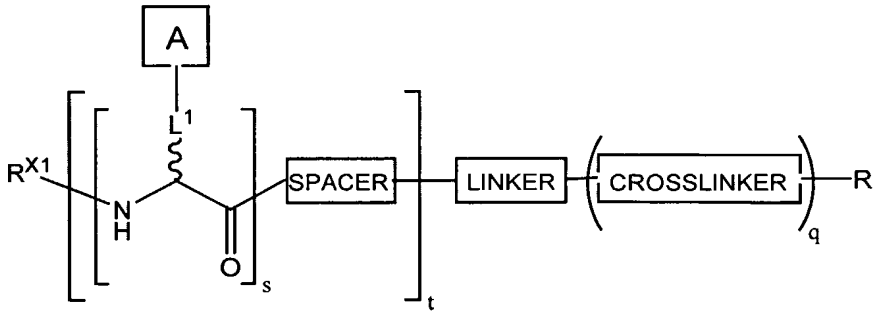
each occurrence of L^1 is independently a substituted or unsubstituted aliphatic or heteroaliphatic moiety; and

each occurrence of A is independently a carbohydrate domain of formula **(I^{det}), (II^{det})** or **(III^{det})**.

[0098] In certain other embodiments, for the clustered multi-antigenic construct described directly above, each occurrence of L^1 is independently $-O(CHR^{aa})_n-$ or –

$NHC(=O)(CHR^{aa})_n$- wherein each occurrence of n is independently an integer from 1-10; and each occurrence of R^{aa} is hydrogen, lower alkyl, aryl, heteroaryl, -alkyl(aryl) or –alkyl(heteroaryl). In certain exemplary embodiments, each occurrence of n is 1 and each occurrence of R^{aa} is hydrogen or methyl. In certain embodiments, each occurrence of L^1 is independently a moiety having the structure $–O(CH_2)_n$- wherein n is an integer from 1-10 and each occurrence of A is O-linked to the construct backbone. In certain embodiments, each occurrence of L^1 is independently a moiety having the structure $–NHC(=O)(CH_2)_n$- wherein n is an integer from 1-10 and each occurrence of A is N-linked to the construct backbone.

[0099] In certain embodiments, for the clustered multi-antigenic constructs described directly above, the structural unit SU, for each occurrence, is independently an amino acid residue, a peptidyl moiety, a bivalent aryl or heteroaryl moiety or a substituted or unsubstituted C_{1-6}alkylidene or C_{2-6}alkenylidene chain wherein up to two non-adjacent methylene units are independently optionally replaced by CO, CO_2, COCO, $CONR^{Z1}$, $OCONR^{Z1}$, $NR^{Z1}NR^{Z2}$, $NR^{Z1}NR^{Z2}CO$, $NR^{Z1}CO$, $NR^{Z1}CO_2$, $NR^{Z1}CONR^{Z2}$, SO, SO_2, $NR^{Z1}SO_2$, SO_2NR^{Z1}, $NR^{Z1}SO_2NR^{Z2}$, O, S, or NR^{Z1}; wherein each occurrence of R^{Z1} and R^{Z2} is independently hydrogen, alkyl, heteroalkyl, aryl, heteroaryl or acyl. In certain embodiments, each occurrence of the structural unit SU is an amino acid residue, and the clustered multi-antigenic construct has the structure:



wherein q is 0 or 1;

each occurrence of s is independently an integer from 2-20;

t is an integer from 1-6;

R^{X1} is hydrogen, alkyl, acyl, aryl, heteroaryl, -alkyl(aryl), -alkyl(heteroaryl) or a nitrogen protecting group;

R is hydrogen or an immunogenic carrier;

each occurrence of the spacer is independently a substituted or unsubstituted aliphatic, heteroaliphatic, aryl, heteroaryl or peptidic moiety;

the linker is either a free carboxylic acid, –O-, (carboxamido)alkyl carboxamide, MBS, primary carboxamide, mono- or dialkyl carboxamide, mono- or diarylcarboxamide, linear or branched chain (carboxy)alkyl carboxamide, linear or branched chain (alkoxycarbonyl)alkyl-carboxamide, linear or branched chain (carboxy)arylalkylcarboxamide, linear or branched chain (alkoxycarbonyl)alkylcarboxamide, an oligoester fragment comprising from 2 to about 20 hydroxy acyl residues, a peptidic fragment comprising from 2 to about 20 amino acyl residues, or a linear or branched chain alkyl or aryl carboxylic ester;

each occurrence of L^1 is independently a substituted or unsubstituted aliphatic or heteroaliphatic moiety; and

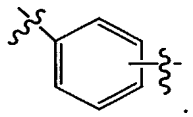
each occurrence of A is independently a carbohydrate domain of formula **(I^{det})**, **(II^{det})** or **(III^{det})**.

[0100] In certain other embodiments, for the two clustered multi-antigenic constructs described directly above, t is ≥ 2 and within each bracketed structure s, independently, each occurrence of A is the same. In certain embodiments, occurrences of A from one bracketed structure s to the next may be the same or different. In certain embodiments, occurrences of A from one bracketed structure s to the next are different. In certain other embodiments, each occurrence of A is independently O or N-linked to the construct backbone. In certain other embodiments, each occurrence of A is independently α- or β-linked to the construct backbone.

[0101] In certain embodiments, for the clustered multi-antigenic construct described directly above, R^{X1} is an acyl moiety. In certain exemplary embodiments, R^{X1} is an amino acid residue.

[0102] In certain embodiments, for the two clustered multi-antigenic constructs described directly above, the spacer, for each occurrence, is independently a substituted or unsubstituted C_{1-6}alkylidene or C_{2-6}alkenylidene chain wherein up to two non-adjacent methylene units are independently optionally replaced by CO, CO_2, COCO, $CONR^{Z1}$, $OCONR^{Z1}$, $NR^{Z1}NR^{Z2}$, $NR^{Z1}NR^{Z2}CO$, $NR^{Z1}CO$, $NR^{Z1}CO_2$, $NR^{Z1}CONR^{Z2}$, SO, SO_2, $NR^{Z1}SO_2$, SO_2NR^{Z1}, $NR^{Z1}SO_2NR^{Z2}$,

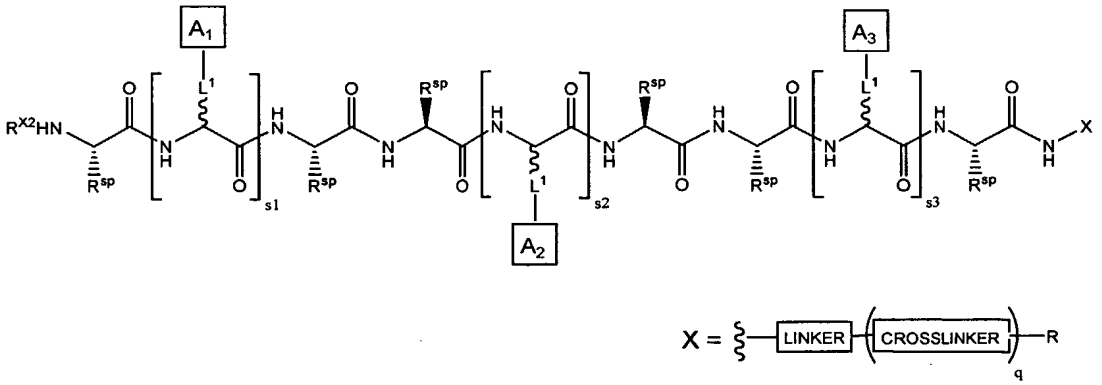
O, S, or NR^{Z1}; wherein each occurrence of R^{Z1} and R^{Z2} is independently hydrogen, alkyl, heteroalkyl, aryl, heteroaryl or acyl; a peptidyl moiety or a bivalent aryl or heteroaryl moiety. In certain exemplary embodiments, the spacer, for each occurrence, is independently $-(CHR^{sp})_n-$, where n is 1-8 and each occurrence of R^{sp} is independently hydrogen, alkyl, cycloalkyl, aryl, heteroaryl, -alkyl(aryl), -alkyl(heteroaryl), $-OR^{sp1}$, $-SR^{sp}1$ or $-NR^{sp1}R^{sp2}$ where R^{sp1} and R^{sp1} are independently hydrogen or lower alkyl; a peptidyl moiety comprising one or more α-amino acid residues, or a bivalent aryl moiety having the structure:



In certain exemplary embodiments, each occurrence of the spacer is independently a dipeptidyl moiety.

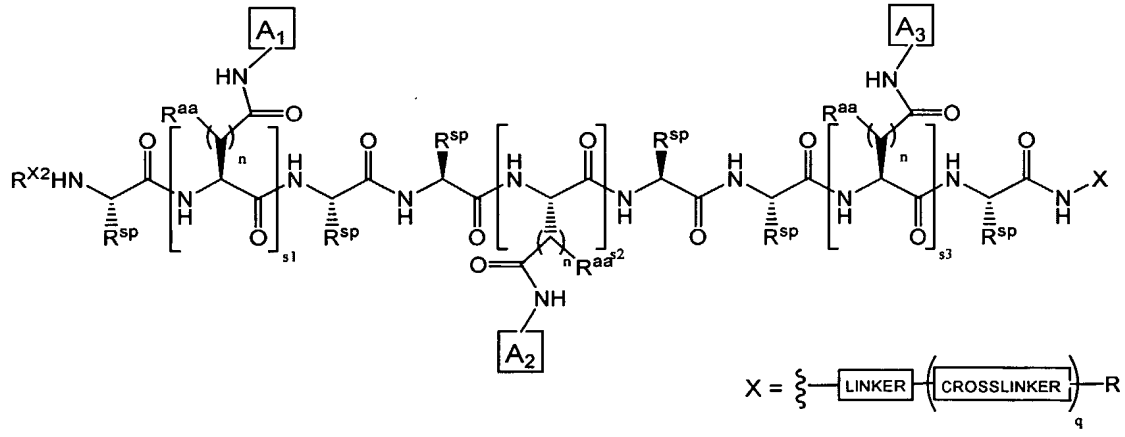
[0103] In certain other embodiments, for the clustered multi-antigenic constructs described directly above, each occurrence of L^1 is independently a natural amino acid side chain. In certain embodiments, each occurrence of L^1 is independently an unnatural amino acid side chain. In certain embodiments, each occurrence of L^1 is independently $-O(CHR^{aa})_n-$ or $-NHC(=O)(CHR^{aa})_n-$ wherein each occurrence of n is independently an integer from 1-10; and each occurrence of R^{aa} is hydrogen, lower alkyl, aryl, heteroaryl, -alkyl(aryl) or –alkyl(heteroaryl). In certain exemplary embodiments, each occurrence of n is 1 and each occurrence of R^{aa} is hydrogen or methyl. In certain embodiments, each occurrence of L^1 is independently a moiety having the structure $-O(CH_2)_n-$ wherein n is an integer from 1-10 and each occurrence of A is O-linked to the construct backbone. In certain embodiments, each occurrence of L^1 is independently a moiety having the structure $-NHC(=O)(CH_2)_n-$ wherein n is an integer from 1-10 and each occurrence of A is N-linked to the construct backbone.

[0104] In certain embodiments, the clustered multi-antigenic constructs described directly above have the following structure:



where the peptide backbone may be linear, as shown, above, or cyclic (e.g., the two occurrences of Rsp at the N- and C-termini, taken together, form a cyclic moiety);

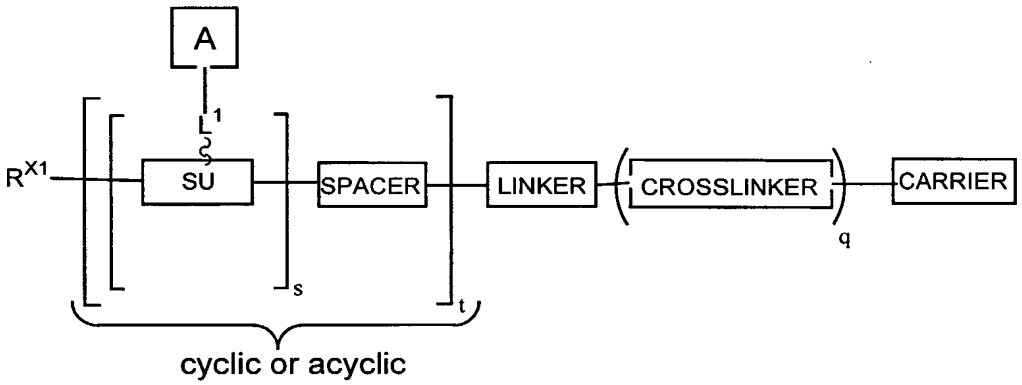
wherein L^1 and R^{sp} are as defined above; s1, s2 and s3 are independently integers from 2-5; A_1-A_3 are independently a carbohydrate domain of formula (**I^{det}**), (**II^{det}**) or (**III^{det}**), and are different from each other; and R^{X2} is hydrogen, alkyl, acyl, aryl, heteroaryl, -alkyl(aryl), -alkyl(heteroaryl) or a nitrogen protecting group. In certain exemplary embodiments, each occurrence of L^1 is independently a natural amino acid side chain. In certain embodiments, each occurrence of L^1 is independently an unnatural amino acid side chain. In certain other embodiments, each occurrence of L^1 is independently $-NHC(=O)(CHR^{aa})_n-$ and the glycopeptide has the structure:



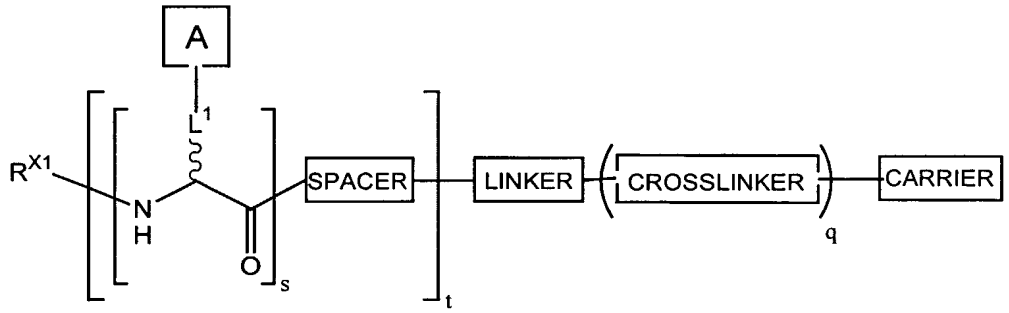
wherein R, R^{X2}, R^{sp}, s1, s2 and s3 and A_1-A_3 are as defined above; each occurrence of n is independently an integer from 1-10; and each occurrence of R^{aa} is hydrogen, lower alkyl, aryl, heteroaryl, -alkyl(aryl) or –alkyl(heteroaryl). In certain

exemplary embodiments, each occurrence of n is 1 and each occurrence of R^{aa} is hydrogen. In certain embodiments, each occurrence of R^{sp} is independently a natural amino acid side chain. In certain exemplary embodiments, each occurrence of R^{sp} is hydrogen.

[0105] In certain embodiments, the clustered multi-antigenic construct is attached to a suitable immunogenic carrier via a linker and the construct has the structure:

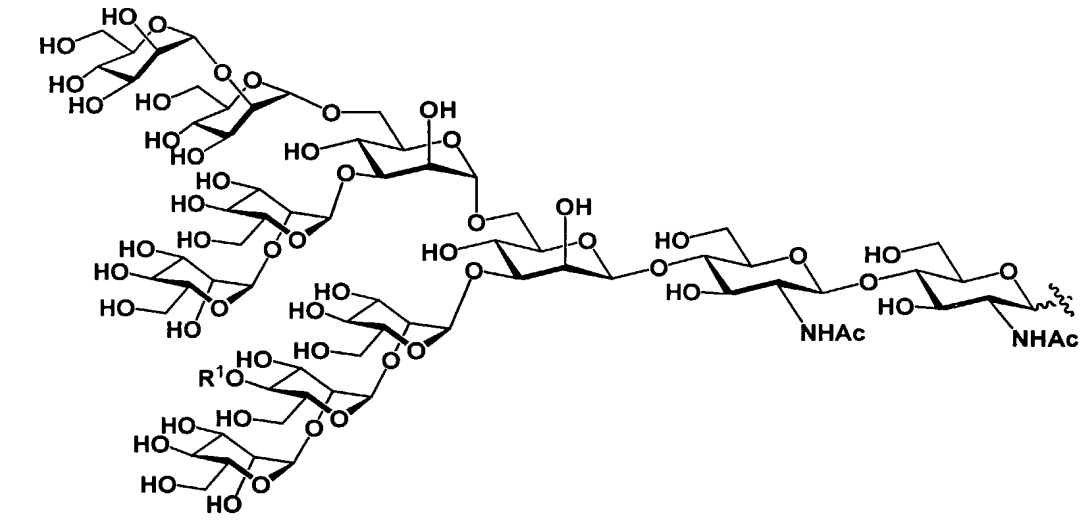


[0106] In certain embodiments, each occurrence of SU is an amino acid residue and the clustered multi-antigenic construct a glycopeptide having the structure:

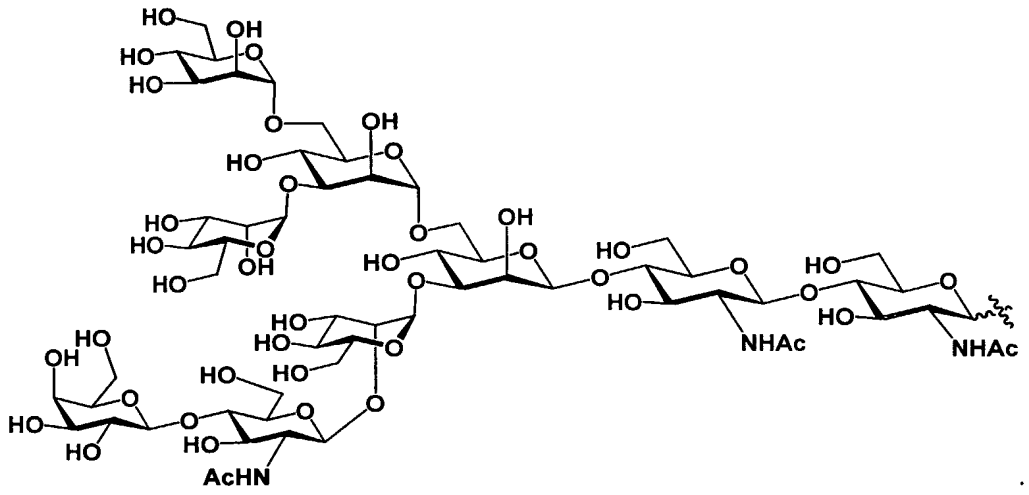


In certain embodiments, for the clusters glycopeptides described above and herein, R is a protein, peptide or lipid immunogenic carrier.

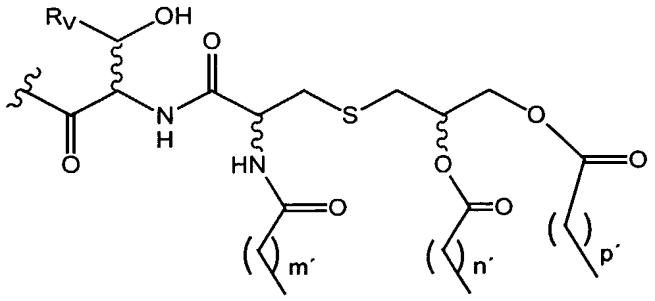
[0107] In certain embodiments, for the clustered multi-antigenic constructs described above and herein, each occurrence of A, A^1, A^2 and A^3 is independently a carbohydrate domain having one of the following structures:



or

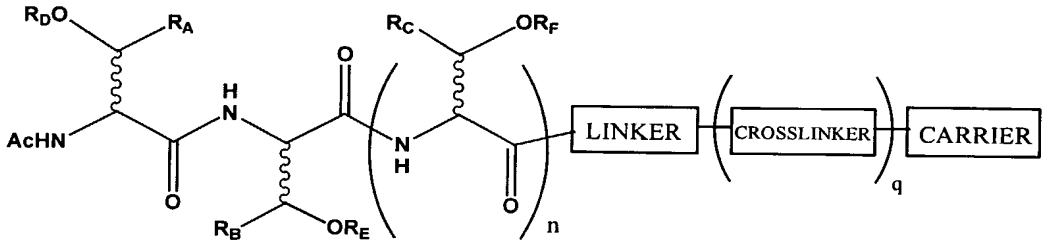


[0108] In certain embodiments, for the clustered multi-antigenic constructs described above and herein, R is a protein, peptide or lipid immunogenic carrier. In certain other embodiments of the present invention, R is NHR'", and the carrier R'" is KLH or Bovine Serum Albumin. In still other embodiments of the present invention, R is NHR'", and the carrier R'" is a lipid having the structure:



wherein m′, n′ and p′ are each independently integers between about 8 and 20; and R_V is hydrogen, substituted or unsubstituted linear or branched chain lower alkyl or substituted or unsubstituted phenyl. In certain exemplary embodiments, m′, n′ and p′ are each 14 and the lipid is tripalmitoyl-S-glycerylcysteinylserine (*e.g.*, PamCys). It will be appreciated that the protein or lipid can be linked to N or the rest of the construct either directly or through a crosslinker and thus R'" incorporates proteins, peptides and lipids, as well as (crosslinker-protein), (crosslinker-peptide) and (crosslinker-lipid) moieties. In certain preferred embodiments, the crosslinker is MMCCH (4-(maleimidomethyl) cyclohexane-1-carboxyl hydrazide).

[0109] In certain embodiments, the invention encompasses multi-antigenic constructs having the structure:



wherein the linker is -O-, -NR_G-, -$NR_G(CR_HR_J)_kNR_K$-, $NR_G(CR_HR_J)_kNR_K(C{=}O)(CR_HR_J)_kS$-, -$(CR_HR_J)_kNR_K$-, -$O(CR_HR_J)_kNR_K$-, an oligoester fragment comprising from 2 to about 20 hydroxy acyl residues, a peptidic fragment comprising from 2 to about 20 amino acyl residues, or a linear or branched chain alkyl or aryl carboxylic ester, wherein each occurrence of k is independently 1-5;

wherein each occurrence of R_G, R_H, R_J or R_K is independently hydrogen, a linear or branched, substituted or unsubstituted, cyclic or acyclic alkyl moiety, or a substituted or unsubstituted aryl moiety;

wherein the crosslinker is a moiety derived from a crosslinking reagent capable of

conjugating the carrier with the linker;

wherein the carrier is a peptide, protein or lipid;

wherein n is 1, 2, 3 or 4;

wherein q is 0 or 1;

wherein each occurrence of R_A, R_B and R_C is independently hydrogen, substituted or unsubstituted linear or branched chain lower alkyl or substituted or unsubstituted phenyl; and

wherein each occurrence of R_D, R_E and R_F are each independently a carbohydrate domain of formula **(I^{det})**, **(II^{det})** or **(III^{det})**.

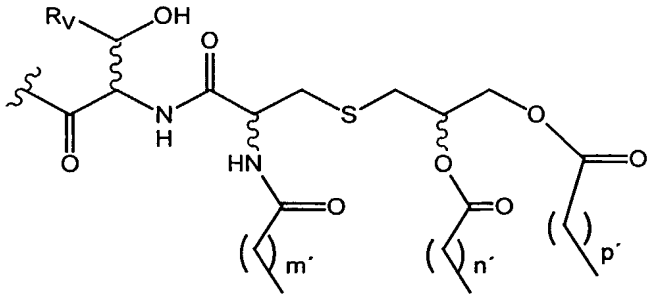
[0110] In certain embodiments, for the multi-antigenic constructs described herein, the linker is -O-, -NR_G-, -NR_G(aliphatic)NR_J-, -NR_G(heteroaliphatic)NR_J-, -(aliphatic)NR_J-, -(heteroaliphatic)NR_J-, -O(aliphatic)NR_J-, -O(heteroaliphatic)NR_J-, -NR_G(aliphatic)NR_J(C=O)$(CR_HR_I)_k$S-, -NR_G(heteroaliphatic)NR_J(C=O)$(CR_HR_I)_k$S-, -(aliphatic)NR_J(C=O)$(CR_HR_I)_k$S-, -(heteroaliphatic)NR_J(C=O)$(CR_HR_I)_k$S-, -O(aliphatic)NR_J(C=O)$(CR_HR_I)_k$S-, -O(heteroaliphatic)NR_J(C=O)$(CR_HR_I)_k$S-, an oligoester fragment comprising from 2 to about 20 hydroxy acyl residues, a peptidic fragment comprising from 2 to about 20 amino acyl residues, or a linear or branched chain alkyl or aryl carboxylic ester, wherein each occurrence of k is independently 1-5; wherein each occurrence of R_G, R_H, R_I or R_J is independently hydrogen, a linear or branched, substituted or unsubstituted, cyclic or acyclic moiety, or a substituted or unsubstituted aryl moiety, and wherein each aliphatic or heteroaliphatic moiety is independently substituted or unsubstituted, linear or branched, cyclic or acyclic.

[0111] In certain embodiments, for the multi-antigenic constructs described herein, the linker is -O-, -$NR_G(CR_HR_I)_kNR_J$-, -$NR_G(CR_HR_I)_kNR_J(C=O)(CR_HR_I)_k$S-, -$NR_G$-, -$(CR_HR_J)_kNR_I$-, -$O(CR_HR_I)_kNR_J$, an oligoester fragment comprising from 2 to about 20 hydroxy acyl residues, a peptidic fragment comprising from 2 to about 20 amino acyl residues, or a linear or branched chain alkyl or aryl carboxylic ester, wherein each occurrence of k is independently 1-5, wherein each occurrence of R_G, R_H, R_I or R_J is independently hydrogen, a linear or branched, substituted or unsubstituted, cyclic or acyclic moiety, or a substituted or unsubstituted aryl moiety.

[0112] In certain embodiments, for the multi-antigenic constructs described herein, the linker is a moiety having the structure –$NH(CH_2)_tNHC(=O)(CH_2)_v$S- wherein t and v are each integers from 1-6. In certain exemplary embodiments, t is 3 and v is 1.

[0113] In certain embodiments, for the multi-antigenic constructs described above, the carrier is a protein, peptide or lipid immunogenic carrier. In certain other

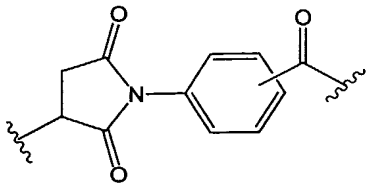
embodiments of the present invention, the carrier is NHR'", and R'" is KLH or Bovine Serum Albumin. In still other embodiments of the present invention, the carrier is NHR'", and R'" is a lipid having the structure:



wherein m', n' and p' are each independently integers between about 8 and 20; and R_V is hydrogen, substituted or unsubstituted linear or branched chain lower alkyl or substituted or unsubstituted phenyl. In certain exemplary embodiments, m', n' and p' are each 14 and the lipid is tripalmitoyl-S-glycerylcysteinylserine (*e.g.*, PamCys). It will be appreciated that the protein or lipid can be linked to N or the rest of the construct either directly or through a crosslinker and thus R'" incorporates proteins, peptides and lipids, as well as (crosslinker-protein), (crosslinker-peptide) and (crosslinker-lipid) moieties. In certain preferred embodiments, the crosslinker is MMCCH (4-(maleimidomethyl) cyclohexane-1-carboxyl hydrazide).

[0114] Crosslinkers suited to the invention are widely known in the art (see, for example, Appendix A: 1994 Pierce Technical Handbook: cross-linking, also available at www.piercenet.com/resources/browse.cfm?fldID=184), including bromoacetic NHS ester, 6-(iodoacetamido)caproic acid NHS ester, maleimidoacetic acid NHS ester, maleimidobenzoic acide NHS ester, etc. In certain preferred embodiments, the crosslinker is MMCCH (4-(maleimidomethyl) cyclohexane-1-carboxyl hydrazide). In certain other preferred embodiments, the crosslinker is MBS (m-maleimidobenzoyl acid N-Hydroxysuccinimidyl ester).

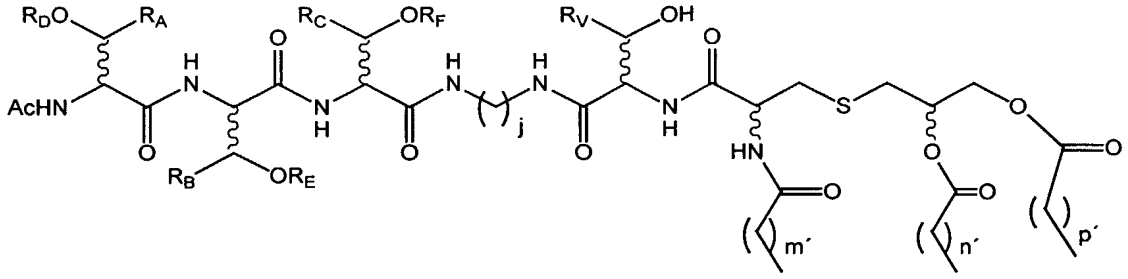
[0115] In certain embodiments, for the multi-antigenic constructs described herein, q is 1 and the crosslinker is a fragment having the structure:



;

[0116] whereby said structure is generated upon conjugation of a maleimidobenzoic acid N-hydroxy succinimide ester with a linker.

[0117] In certain other embodiments, the invention encompasses multi-antigenic constructs having the structure:



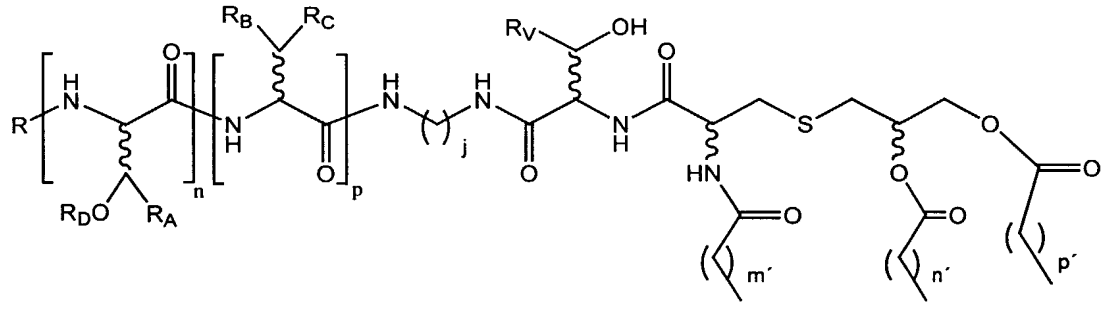
wherein m′, n′ and p′ are integers between about 8 and 20;

j is an integer between 1 and about 8;

R_V, R_A, R_B and R_C are independently hydrogen, substituted or unsubstituted linear or branched chain lower alkyl or substituted or unsubstituted phenyl; and

R_D, R_E and R_F are each independently a carbohydrate domain of formula **(I^{det})**, **(II^{det})** or **(III^{det})**. In certain exemplary embodiments, j is 3.

[0118] In certain other embodiments, the invention encompasses multi-antigenic constructs having the structure:



wherein n and p are each independently an integer from 1-6;

m′, n′ and p′ are independently integers between about 8 and 20;

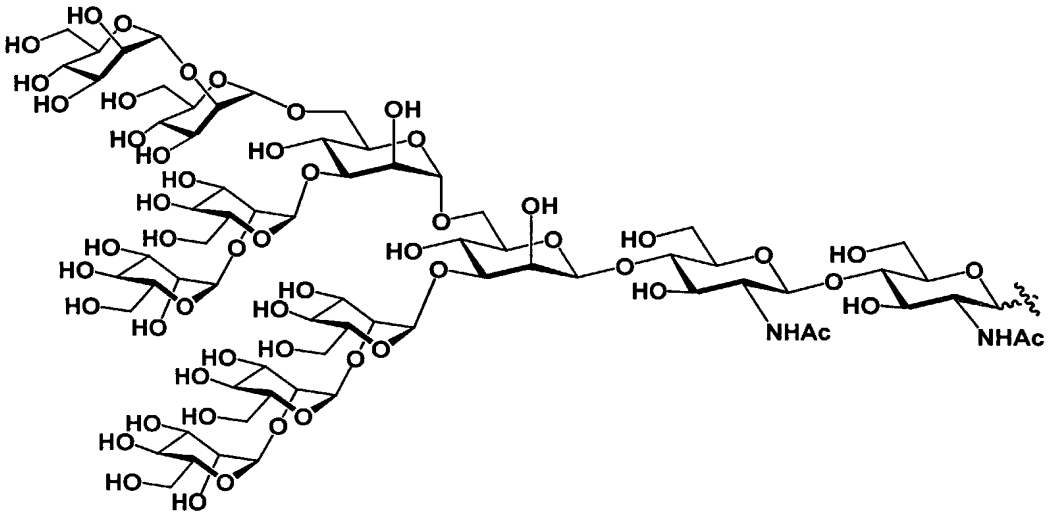
j is an integer between 1 and about 8;

R is a nitrogen protecting group;

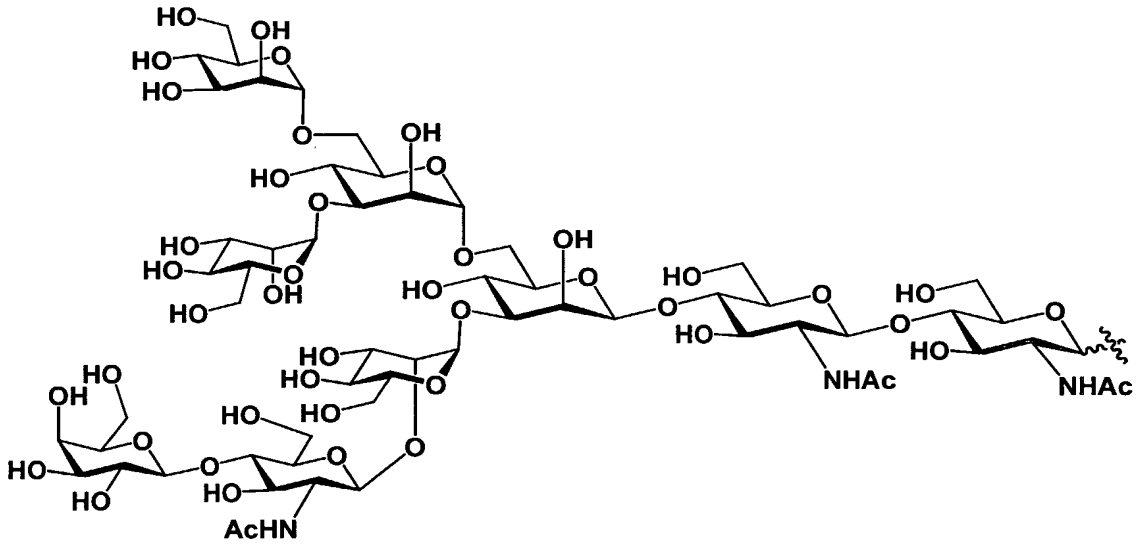
R_V, and R_A, R_B, R_C, R_E and R_F, for each occurrence, are independently hydrogen, substituted or unsubstituted linear or branched lower alkyl or substituted or unsubstituted phenyl;

each occurrence of R_D is independently a carbohydrate domain of formula (I^{det}), (II^{det}) or (III^{det}). In certain exemplary embodiments, j is 3.

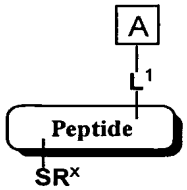
[0119] In certain embodiments, for the clustered multi-antigenic constructs described above and herein, each occurrence of R_D, R_E and R_F is independently a carbohydrate domain having one of the following structures:



or



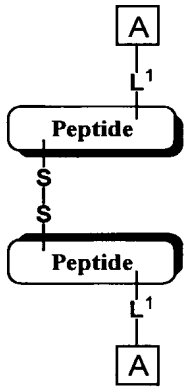
[0120] In certain embodiments, the invention provides glycopeptides comprising one occurrence of a carbohydrate domain of the formula (I^{det}), (II^{det}) or (III^{det}), whereby the glycopeptide structure allows for dimerization. In certain exemplary embodiments, the glycopeptide comprises one cysteine residue and the glycopeptide has the structure:



wherein L^1 is as defined above; A is a carbohydrate domain of the formula **(I^{det})**, **(II^{det})** or **(III^{det})**; R^x is hydrogen or a thiol protecting group.

[0121] The skilled practitioner will know how to select a thiol protecting group to allow protection/deprotection of the cysteine thiol without negatively affecting other protecting groups that might be present on the construct (e.g., on carbohydrate A). Guidance can be found, for example, in "Protective Groups in Organic Synthesis" , Chapter 6, Third Ed. Greene, T.W. and Wuts, P.G., Eds., John Wiley & Sons, New York: 1999, the entire contents of which are hereby incorporated by reference. In certain exemplary embodiments, R^x is–StBu.

[0122] In certain embodiments, inventive constructs comprising one or more carbohydrate domains of the formula **(I^{det})**, **(II^{det})** or **(III^{det})** are dimers of the above glycopeptides, and the constructs have the structure:



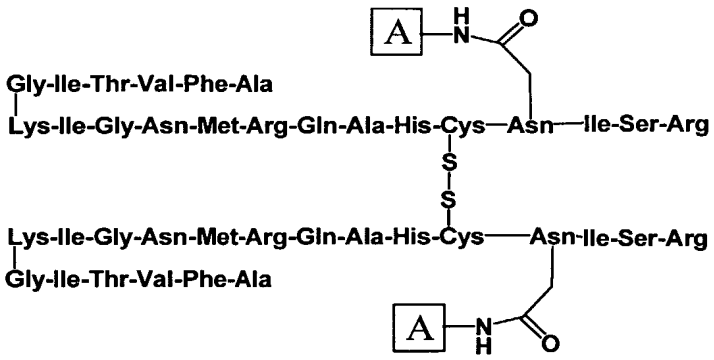
wherein each peptide may be the same or different; and each occurrence of A is independently a carbohydrate domain of the formula **(I^{det})**, **(II^{det})** or **(III^{det})**.

[0123] In certain other embodiments, for the two glycopeptidic constructs described above, each occurrence of L^1 is independently a natural amino acid side chain. In certain embodiments, each occurrence of L^1 is independently an unnatural amino acid side chain. In certain embodiments, each occurrence of L^1 is independently $–O(CHR^{aa})_n-$ or $–NHC(=O)(CHR^{aa})_n-$ wherein each occurrence of n is independently an integer from 1-10; and each occurrence of R^{aa} is hydrogen, lower alkyl, aryl, heteroaryl, -alkyl(aryl) or –alkyl(heteroaryl). In certain exemplary

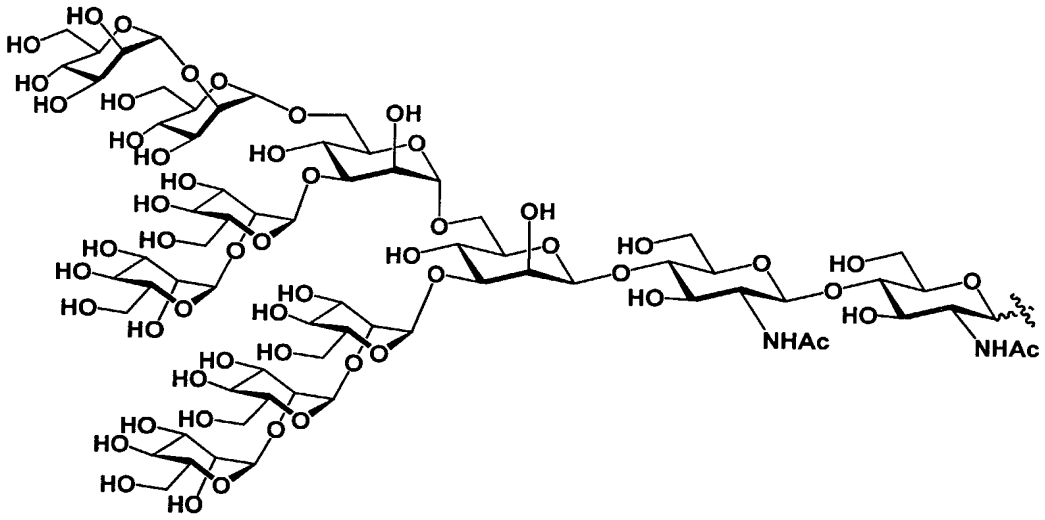
embodiments, each occurrence of n is 1 and each occurrence of R^{aa} is hydrogen or methyl. In certain embodiments, each occurrence of L^1 is independently a moiety having the structure $-O(CH_2)_n-$ wherein n is an integer from 1-10 and each occurrence of A is O-linked to the construct backbone. In certain embodiments, each occurrence of L^1 is independently a moiety having the structure $-NHC(=O)(CH_2)_n-$ wherein n is an integer from 1-10 and each occurrence of A is N-linked to the construct backbone. In certain embodiments, each occurrence of L^1 is an aspartyl side chain.

[0124] In certain embodiments, for the two glycopeptidic constructs described above, the peptide has a structure that is either identical or closely related to that of gp120 near an N-glycosylation site. In certain embodiments, for the two glycopeptidic constructs described above, the peptide comprises the amino acid sequence: Cys-Asn-Ile-Ser-Arg, wherein any one or more of the amino acid residues may bear one or more protecting groups. In certain embodiments, for the two glycopeptidic constructs described above, the peptide comprises the amino acid sequence: Ala-Phe-Val-Thr-Ile-Gly-Lys-Ile-Gly-Asn-Met-Arg-Gln-Ala-His-Cys-Asn-Ile-Ser-Arg, wherein any one or more of the amino acid residues may bear one or more protecting groups.

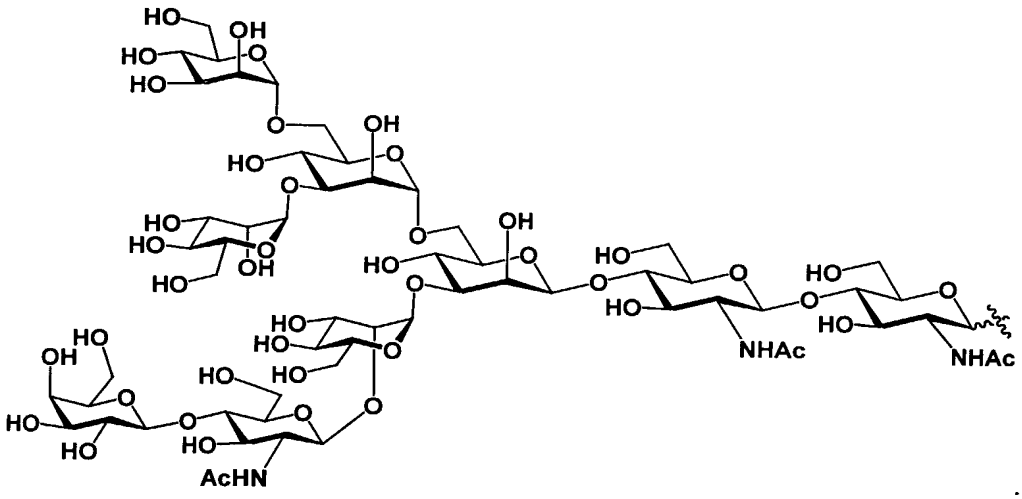
[0125] In certain embodiments, the invention provides dimeric constructs having the structure:



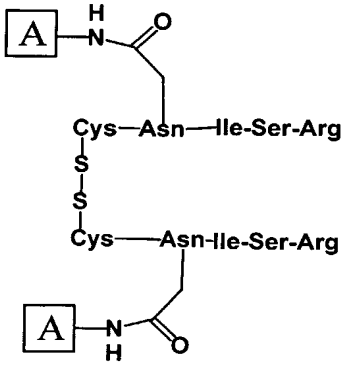
wherein each occurrence of A is independently a carbohydrate domain having one of the structures:



or



[0126] In certain embodiments, dimeric constructs having the following structure are provided:



wherein A is as defined above.

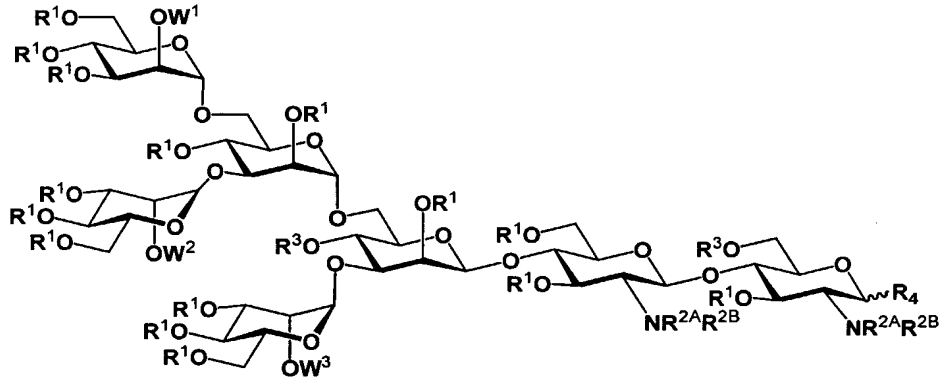
[0127] **2) Synthetic Methodology**

[0128] The practitioner has a a well-established literature of carbohydrate chemistry to draw upon, in combination with the information contained herein, for guidance on synthetic strategies, protecting groups, and other materials and methods useful for the synthesis of the compounds of this invention, and conjugates thereof.

[0129] The various patent documents and other references cited herein provide helpful background information on preparing compounds similar to the inventive compounds described herein or relevant intermediates, as well as information on formulation, uses, and administration of such compounds which may be of interest.

[0130] Moreover, the practitioner is directed to the specific guidance and examples provided in this document relating to various exemplary compounds and intermediates thereof.

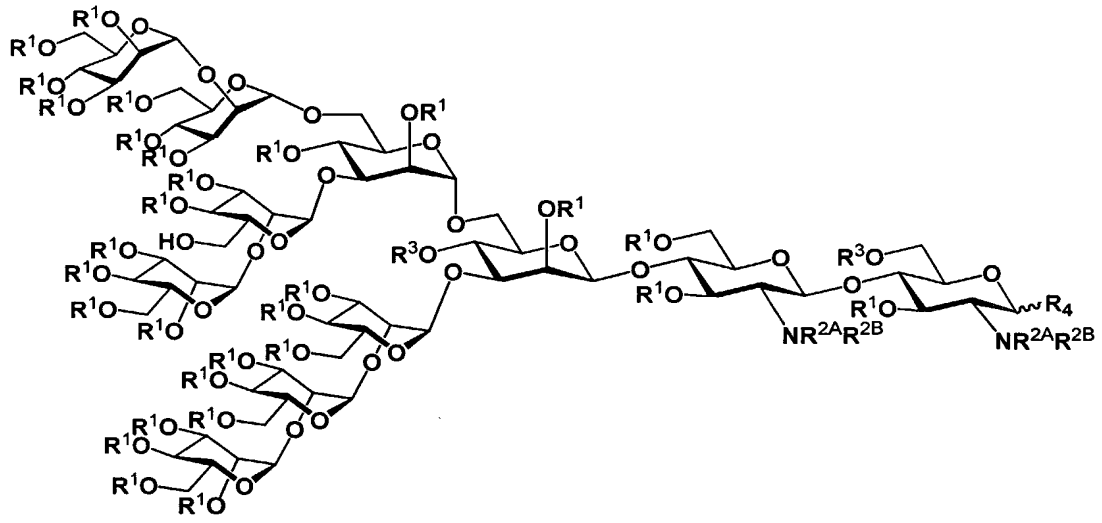
[0131] In one aspect of the invention, there is provided a method for preparing isolated an compound of formula **(I)**:



(I)

[0132] wherein R^1, R^{2A}, R^{2B}, R^3, R^4 and W^1-W^3 are as defined generally above and in classes and subclasses herein.

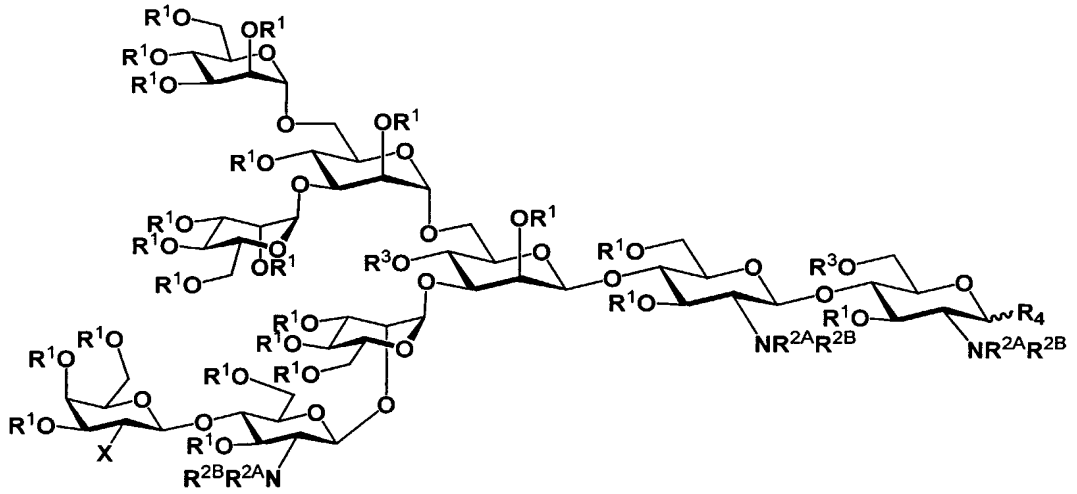
[0133] In one aspect of the invention, there is provided a method for preparing an isolated compound of formula **(II)**:



(II)

wherein R^1, R^{2A}, R^{2B}, R^3 and R^4 are as defined generally above and in classes and subclasses herein.

[0134] In another aspect of the invention, there is provided a method for preparing isolated an compound of formula **(III)**:



(III)

[0135] wherein X, R^1, R^{2A}, R^{2B}, R^3 and R^4 are as defined generally above and in classes and subclasses herein.

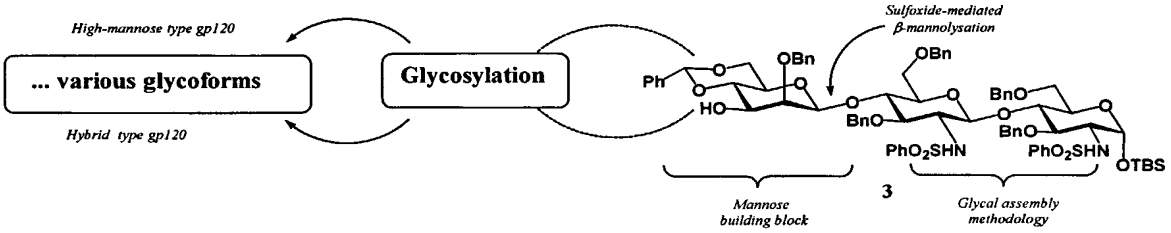
[0136] In certain exemplary embodiments, R^4 is $-NHR^{4A}$; wherein R^{4A} is an amino acyl residue of a peptide and the invention provides a method for preparing homogeneous N-linked gp120-derived glycopeptides.

[0137] ***Glycan Synthesis***

[0138] Glycan synthesis generally suffers from the stereochemical diversity of its targets and therefore of its building blocks, as well. The advent of a new target often requires a reworked, if not entirely different synthetic plan, based on varying protecting groups, coupling strategies, and starting materials. The present invention provides a method allowing access to a number of gp120-derived saccharides using only a small set of building blocks and the same general procedure for each glycan.

[0139] In certain embodiments, trisaccharide **3** in Scheme 1 embodies the protected core structure reported for the glycoforms expressed in gp120.

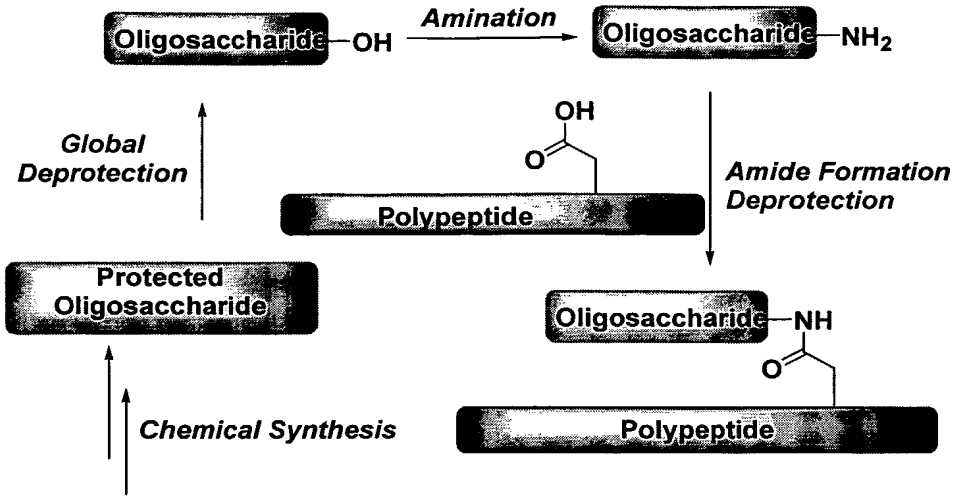
[0140] **Scheme 1.** Proposed methodology for glycan synthesis.



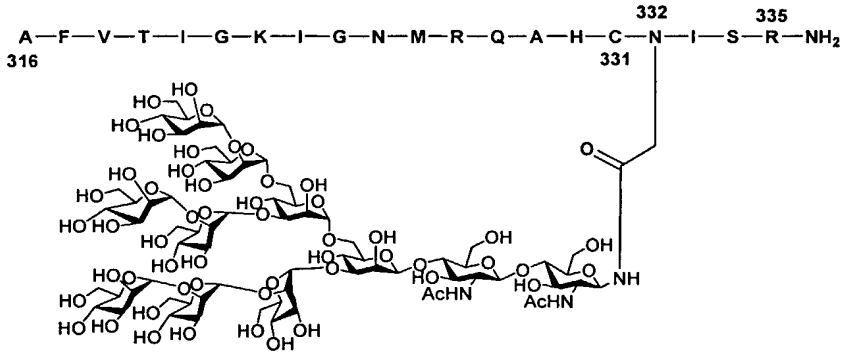
[0141] In certain exemplary embodiments, trisaccharide **3** may be elaborated to give a pentasaccharide either by deprotection of the 6-position followed by simultaneous α-mannosylation at the free 3- and 6-positions or by sequential mannosylation at the 3-and 6-positions with an intermediate deprotection step. Simultaneous mannosylation with equivalently protected mannosyl donors would yield a "symmetrically" substituted pentasaccharide; further deprotections and glycosylations could be achieved in a synchronous fashion at both nonreducing termini. Sequential mannosylation would allow the inclusion of differentially protected mannose building blocks, permitting independent elaboration of the 3- and 6-substituted antennae. Thus the high-mannose pentasaccharide core (which is conserved in most natural *N*-linked glycans) may be synthesized in large quantities and used as a starting point for all of the gp120 targets. Moreover, because hybrid-type gp120 differs from high-mannose type gp120 in its degree of branching beyond the core pentasaccharide, this synthetic scheme would provide easy access to the multi-antennary glycoforms expressed in gp120.

[0142] In certain embodiments, the synthetic approach includes: synthesis of protected oligasaccaride (undecassaccharide), global deprotection to prepare free glycan, amination, coupling with peptide acid and deprotection (Scheme 2).

[0143] **Scheme 2.** Exemplary synthetic strategy

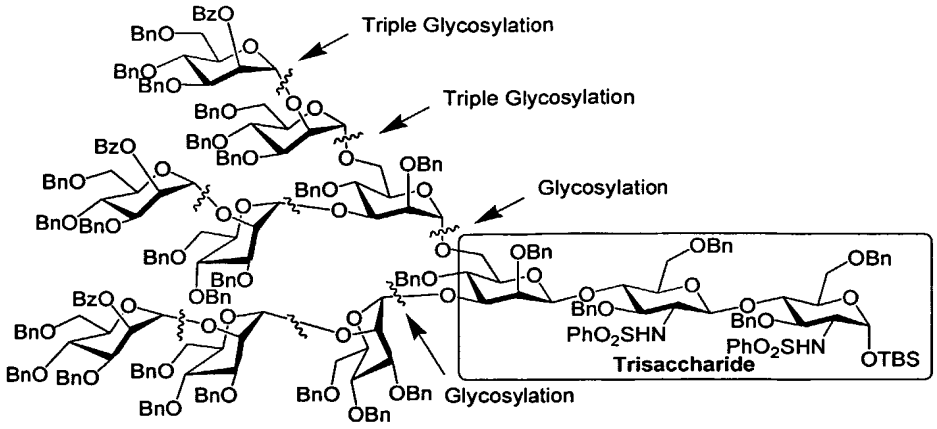


[0144] In certain embodiments, a synthesis for the high-mannose type glycopeptide having the structure:



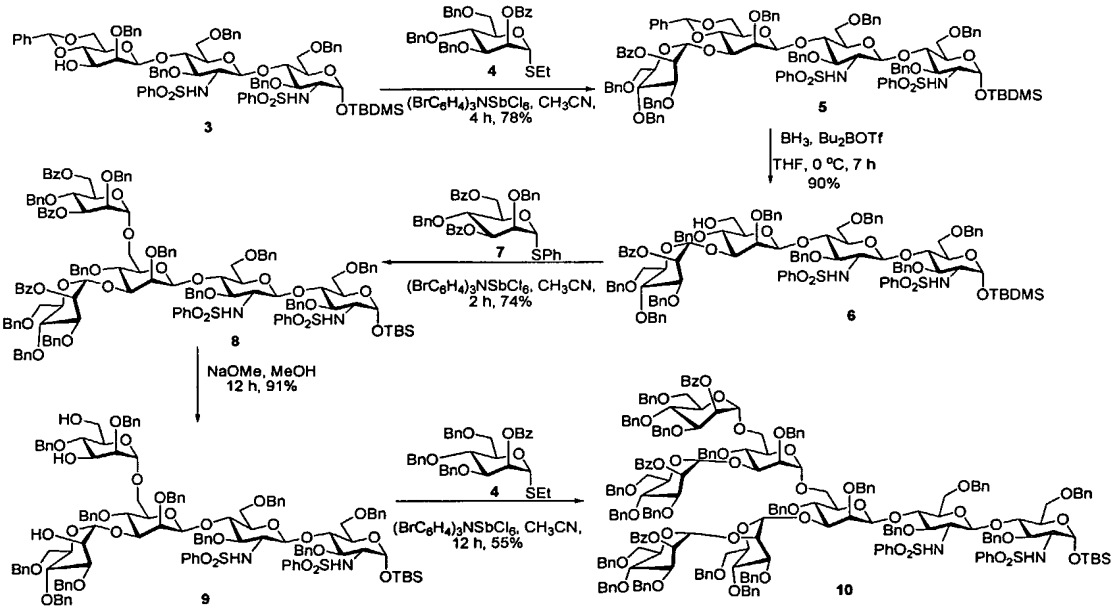
is provided. In certain embodiments, a synthetic plan for the preparation of the undecasaccharide is shown in Scheme 3. For example, starting from a trisaccharide intermediate (e.g., trisacchraide **3**),[1] two successive glycosylations will give pentasaccharide, then two consecutive triple glycosylation would furnish the undecasaccharide.

[0145] **Scheme 3.** Exemplary retrosynthesis of undesaccharide **1**.

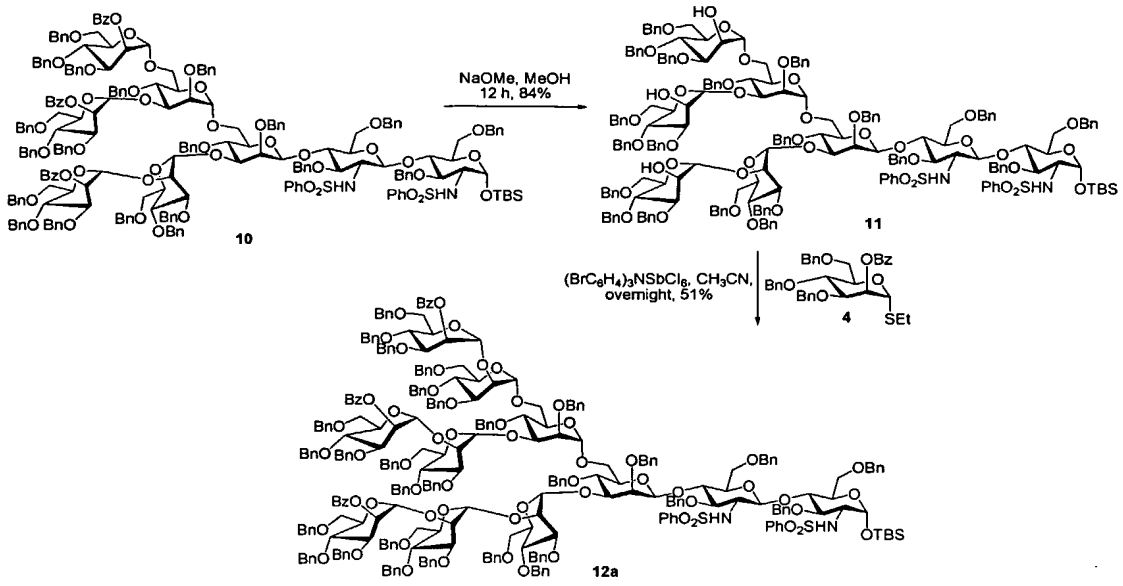


[0146] An exemplary synthesis using this route is shown in Scheme 4. For example, mannosylation of trisaccharide **3** using thiomannoside **4** and Sinaÿ radical cation activation[2,3] gave tetresaccharide in 78% yield. The benzylidene ring was reductively opened by borane and the resulting free alcohol **5** underwent mannosylation to give pentasaccharide **8** in 74% yield. After Zemplen reaction, the newly generated three free OH were mannosylated to afford octasaccharide **10** using same Sinaÿ conditions[2,3]. The same triple-glycosylation sequence was repeated to synthesize the undecsaccharide **12a** in 55% yield (Scheme 5).

[0147] **Scheme 4**

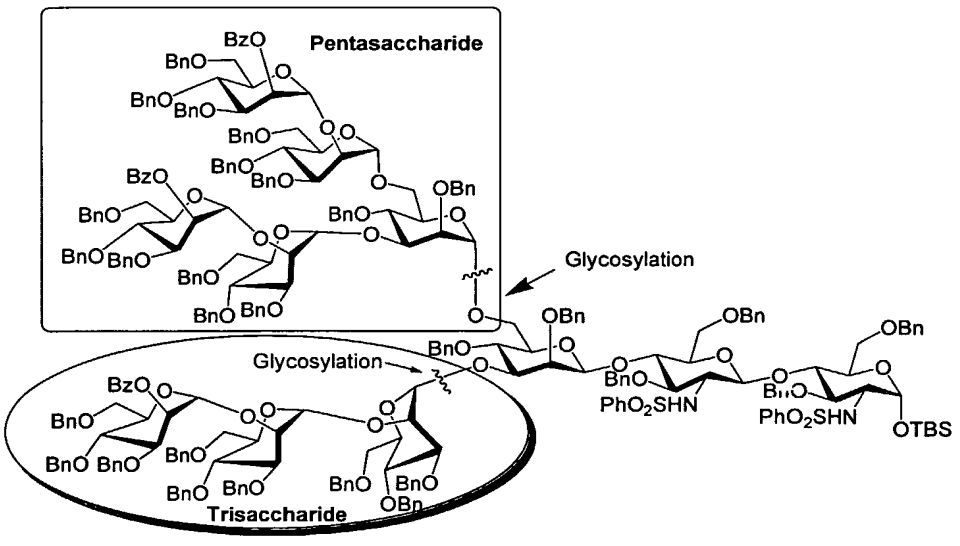


[0148] **Scheme 5**



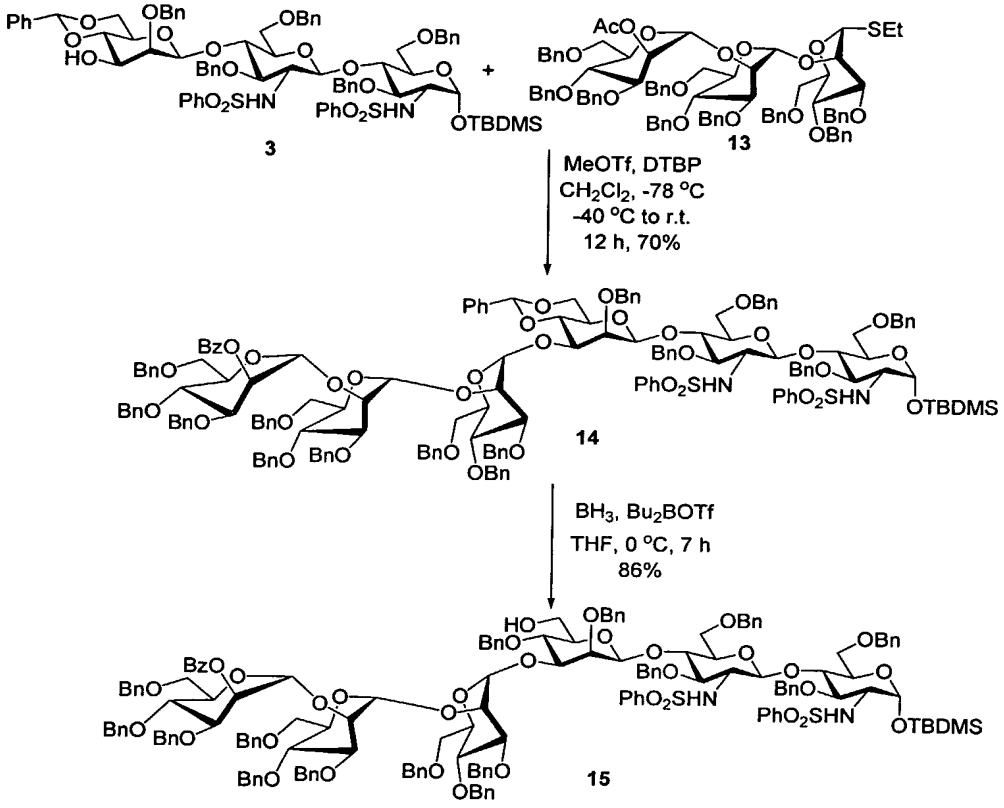
[0149] For example, as shown in Scheme 6, the desired undecasaccharide could be synthesized by a 3+3 glycosylation (trisaccharide couples with another trisaccharide) followed by a 6+5 coupling. This synthetic plan is much shorter and more convergent than the first strategy.

[0150] **Scheme 6**



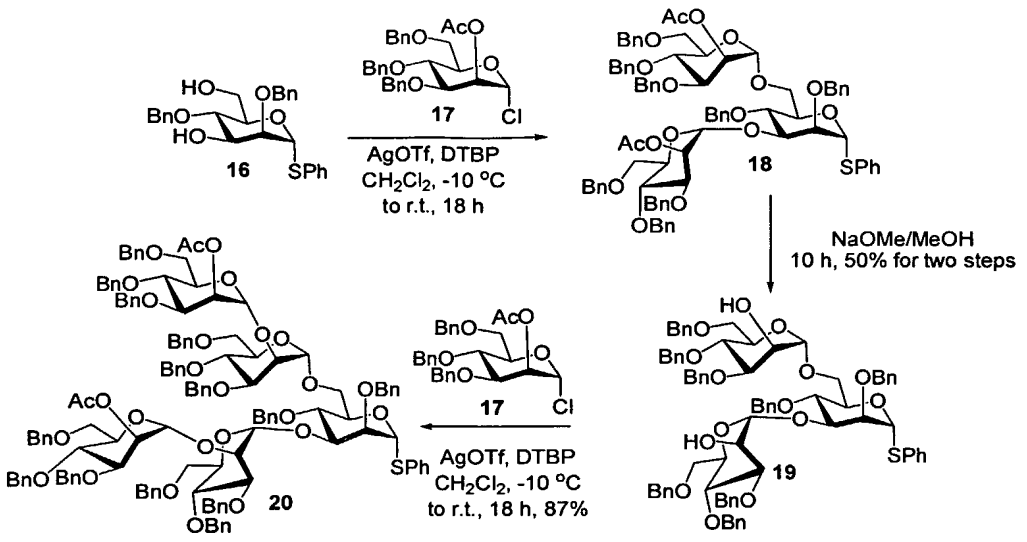
[0151] In certain embodiments, as shown in scheme 7, trisaccharide **3** first underwent glycosylation with trisaccharide donor **13** using MeOTf as promoter to afford hexasaccharide in 70% yield. Then reductive ring-opening of the benzylidene ring gave saccharide **15** in 87% yield.

[0152] **Scheme 7**



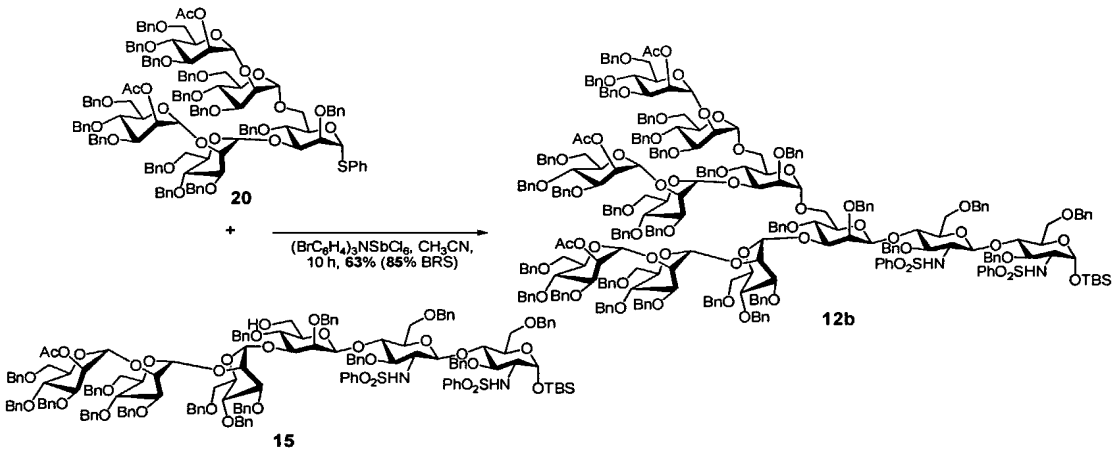
[0153] In certain embodiments, the pentasaccharide which is the precursor for the upper-left portion of the final compound **(1)** was synthesized as shown in scheme 8. For example, double-glycosylation of mannose derivative **16** using chloro donor **17** and promoter silver triflate gave trisaccharide **18**. After cleavage of the two acetyl groups, another double-glycosylation provided pentasaccharide **20** in 87% yield.

[0154] **Scheme 8**



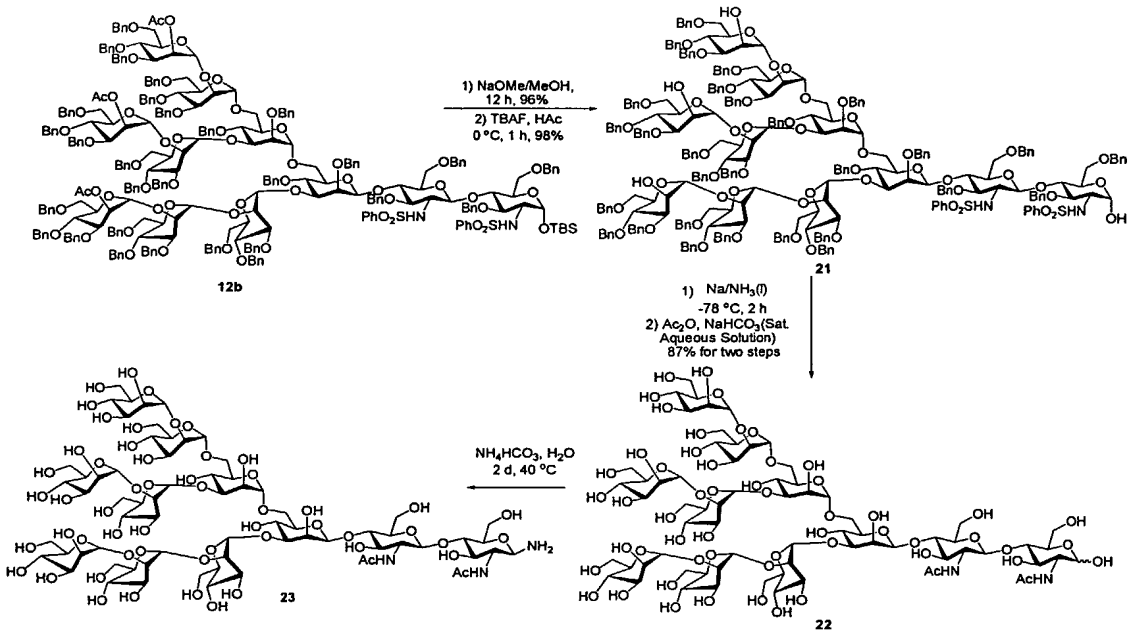
[0155] In certain embodiments, the 6+5 glycosylation using Sinaÿ radical cation activation[2,3] proceeded smoothly giving the desired undecasaccharide **12b** in 85% yield (Scheme 9).

[0156] **Scheme 9**



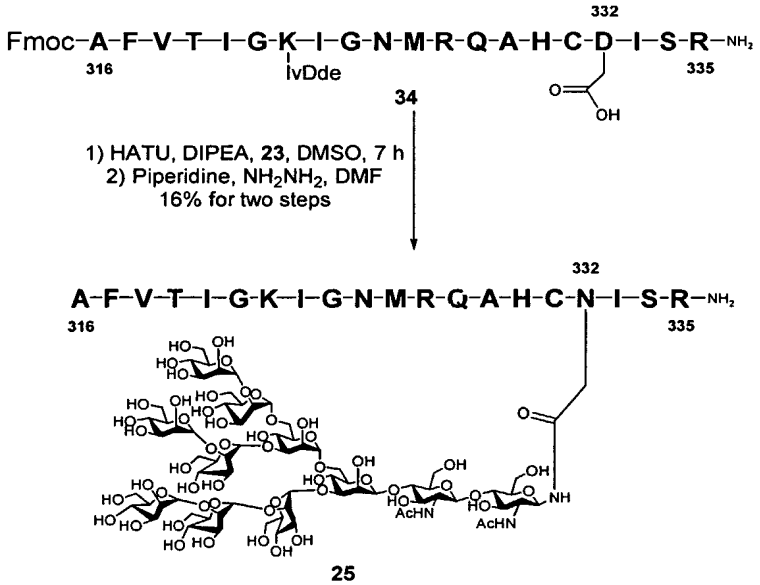
[0157] In certain embodiments, protected undecasaccharide **12b** was treated with sodium methoxide and HF-pyridine to remove the acetyl groups and TBS group, respectively. The resulting oligosaccharide **21** was then subjected to global Birch deprotection followed by selective acetylation using acetyl anhydride in saturated sodium bicarbonate solution to give free glycan in high yield.[5] Following Kochetkov amination[6] furnished free glycosylamine (Scheme 10).

[0158] **Scheme 10**



[0159] In certain embodiments, 20-mer peptide acid **34**, which was made through applied biosynthesis synthesizer, was activate using HATU and coupled directly with glycosylamine **23**. The Fmoc and ivDde protecting groups were removed by treatment with hydrazine and piperidine to give glycopeptide fragment **25** in 16% two steps yield (Scheme 11).

[0160] **Scheme 11**



[0161] Methods of preparing trisaccharide 3 are known in the art. For example, guidance may be found in U.S. Provisional Patent Application No.: 60/500,161 filed September 4, 2003; and International Application No.: PCT/US03/ , filed December 3, 2003 entitled "Prostate Specific Antigens, Conjugates Thereof, Methods for their Preparation and Uses Thereof"; the entire contents of each of the above applications are hereby incorporated by reference herein.

[0162] **References** ("Glycan synthesis" section)

[0163] **1.** Dudkin, V. Y.; Miller, J. S.; Danishefsky, S. J. Tetrahedron Letters **2003**, 44, 1791-1793.

[0164] **2.** Zhang, Y.-M.; Mallet, J.-M.; Sinay, P. *Carbohydrate Research* **1992**, *236*, 73-88.

[0165] **3.** Marra, A.; Mallet, J. M.; Amatore, C.; Sinay, P. *Synlett* **1990**, 572-574.

[0166] **4.** Matsuo, I.; Wada, M.; Manabe, S.; Yamaguchi, Y.; Otake, K.; Kato, K.; Ito, Y. *Journal of the American Chemical Society* **2003**, *125*, 3402-3403.

[0167] **5.** Calarese, D. A.; Scanlan, C. N.; Zwick, M. B.; Deechongkit, S.; Mimura, Y.; Kunert, R.; Zhu, P.; Wormald, M. R.; Stanfield, R. L.; Roux, K. H.; Kelly, J. W.; Rudd, P. M.; Dwek, R. A.; Katinger, H.; Burton, D. R.; Wilson, I. A. *Science (Washington, DC, United States)* **2003**, *300*, 2065-2071.

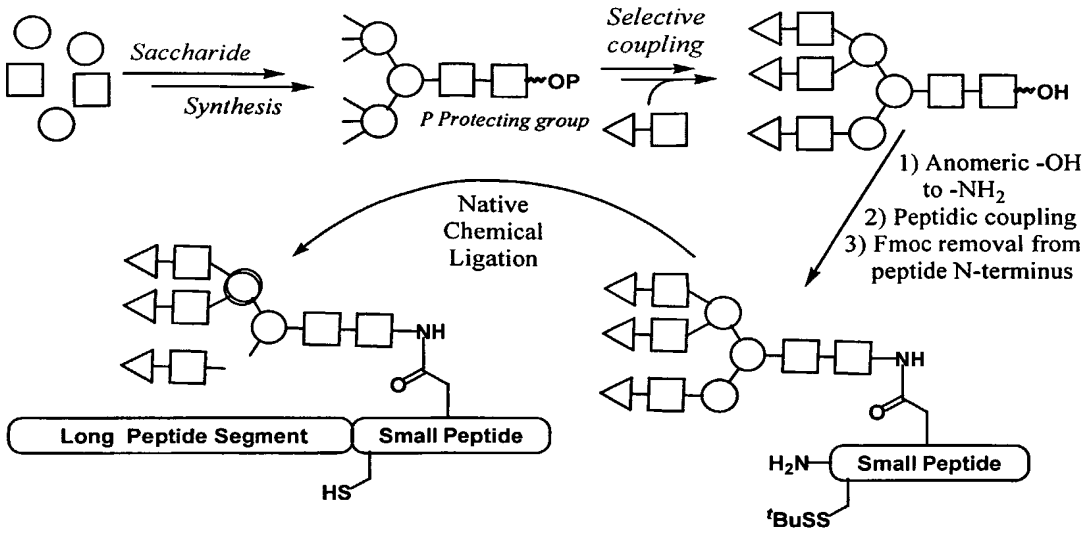
[0168] **6.** Likhosherstov, L. M.; Novikova, O. S.; Derevitskaya, V. A.; Kochetkov, N. K. *Carbohydrate Research* **1986**, *146*, C1-C5.

[0169] ***Glycopeptides***

[0170] Automated peptide synthesis is reliable for sequences up to about 60 amino acid residues in length, but saccharide moieties contained in glycopeptides render their solid phase synthesis less practical. Unlike peptide synthesis, complex glycan and glycoconjugate synthesis remains readily accessible only to a few select laboratories (See, for example, Hang, H. C.; Bertozzi, C. R. "Chemoselective approaches to glycoprotein assembly." *Acc. Chem. Res.* **2001**, *34*, 727-736). Syntheses of several natural *O*-linked glycopeptides containing simple glycans have been reported (See, for example, (1) Arsequell, G.; Haurum, J. S.; Elliott, T.; Dwek, R. A.; Lellouch, A. C. "Synthesis of Major Histocompatibility Complex Class-I

Binding Glycopeptides." *J. Chem. Soc.-Perkin Trans. 1* **1995**, 1739-1745, (2) Chen, X. T.; Sames, D.; Danishefsky, S. J. "Exploration of modalities in building alpha-O-linked systems through glycal assembly: A total synthesis of the mucin-related F1 alpha antigen." *J. Am. Chem. Soc.* **1998**, *120*, 7760-7769; (3) Macmillan, D.; Bertozzi, C. R. "New directions in glycoprotein engineering." *Tetrahedron* **2000**, *56*, 9515-9525; (4) Koeller, K. M.; Smith, M. E. B.; Huang, R. F.; Wong, C. H. "Chemoenzymatic synthesis of a PSGL- 1 N-terminal glycopeptide containing tyrosine sulfate and alpha-O-linked sialyl Lewis X." *J. Am. Chem. Soc.* **2000**, *122*, 4241-4242; (5) Ajisaka, K.; Miyasato, M.; Ishii-Karakasa, I. "Efficient synthesis of O-linked glycopeptide by a transglycosylation using endo alpha-N-acetylgalactosaminidase from Streptomyces sp." *Biosci. Biotechnol. Biochem.* **2001**, *65*, 1240-1243; and (6) Marcaurelle, L. A.; Mizoue, L. S.; Wilken, J.; Oldham, L.; Kent, S. B. H.; Handel, T. M.; Bertozzi, C. R. "Chemical synthesis of lymphotactin: A glycosylated chemokine with a C-terminal mucin-like domain." *Chem. Eur. J.* **2001**, *7*, 1129-1132), as have examples of mimetics for *N*-linked glycopeptides (See, for example, Hang, H. C.; Bertozzi, C. R. "Chemoselective approaches to glycoprotein assembly." *Acc. Chem. Res.* **2001**, *34*, 727-736), and a chemoenzymatic synthesis of an *N*-linked glycopeptide (See, for example, Inazu, T.; Haneda, K.; Mizuno, M. "Synthetic study on N-glycopeptides." *J. Syn. Org. Chem. Jpn.* **1998**, *56*, 210-220), but no chemical synthesis has been reported for a natural *N*-linked glycopeptide with complex glycan and peptide structure. The state of the art for chemically synthesized *N*-linked glycopeptides is exemplified by the pentadecasaccharide *N*-linked to a pentapeptide reported by Wang and coworkers, which was recognized by appropriate antibodies to the H-type blood group antigens present at the glycan nonreducing termini (See, for example, Wang, Z. G.; Zhang, X. F.; Visser, M.; Live, D.; Zatorski, A.; Iserloh, U.; Lloyd, K. O.; Danishefsky, S. J. "Toward fully synthetic homogeneous glycoproteins: A high mannose core containing glycopeptide carrying full H-type2 human flood group specificity." *Angew. Chem. Int. Ed.* **2001**, *40*, 1728-1732).

[0171] **Scheme 12.** Exemplary synthetic approach for the preparation of gp120 glycopeptides.



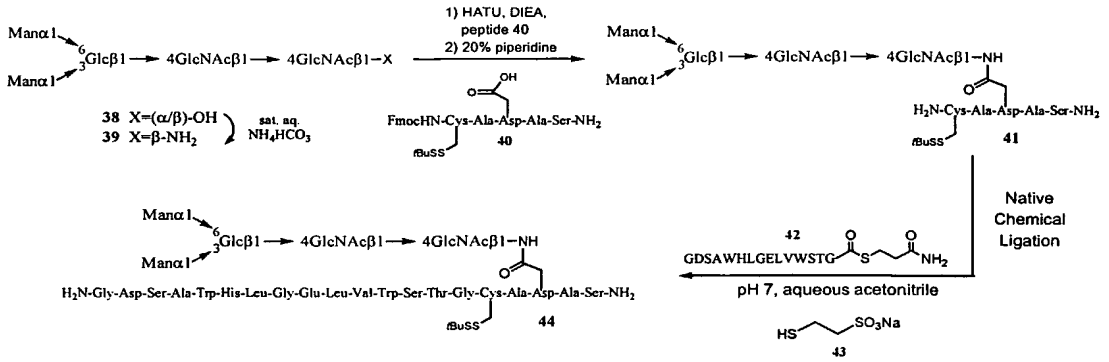
[0172] In certain embodiments, as shown in Scheme 12, the chemical synthesis of inventive glycopeptides may be divided logically into two sections: glycan synthesis (top) and glycopeptide assembly (bottom). At its core, the inventive method would extend the method of Wang, *et al.* (Wang, Z. G.; Zhang, X. F.; Visser, M.; Live, D.; Zatorski, A.; Iserloh, U.; Lloyd, K. O.; Danishefsky, S. J. "Toward fully synthetic homogeneous glycoproteins: A high mannose core containing glycopeptide carrying full H-type2 human flood group specificity." *Angew. Chem. Int. Ed.* **2001**, *40*, 1728-1732) to include one or more peptide elongation steps after synthesis of a short glycopeptide, allowing entry into the realm of fully elaborated, naturally derived glycoproteins (See, for example, Dawson, P. E.; Kent, S. B. H. "Synthesis of native proteins by chemical ligation." *Annu. Rev. Biochem.* **2000**, *69*, 923-960). In an inventive and important improvement, the glycan is fashioned here in a more convergent manner than previously realized, allowing the strategy to be adjusted in its late stage to accommodate the synthesis of various glycoforms, as illustrated in the next section.

[0173] ***Glycopeptide Assembly***

[0174] Guidance for glycopeptide assembly may be found, inter alia, in U.S. Provisional Patent Application No.: 60/500,161 entitled "Prostate Specific Antigens, Conjugates Thereof, Methods for their Preparation and Uses Thereof", filed September 4, 2003; the entire contents of which are hereby incorporated by reference herein. For example, a glycopeptide assembly strategy, as outlined in Scheme 12, involves peptide glycosylation followed by elongation of the peptide

backbone, was examined, as illustrated in Scheme 13, using a model peptide and glycan (Miller, J. S. *et al., Angew. Chemie Int. Ed.*, 2003, **42**, 431). To prepare free glycan **38** for coupling, its anomeric hydroxyl was first aminated to give β-aminoglycoside **39** as described by Kochetkov (See, for example, Likhosherstov, L. M.; Novikova, O. S.; Derevitskaja, V. A.; Kochetkov, N. K. "A New Simple Synthesis of Amino Sugar Beta-D-Glycosylamines." *Carbohydr. Res.* **1986**, *146*, C1-C5). Glycosylamine **39** and the aspartate free acid of peptide **40** were coupled in peptidic fashion according to the procedure of Lansbury and coworkers ((1) Cohen-Anisfeld, S. T.; Lansbury, P. T. "A Practical, Convergent Method for Glycopeptide Synthesis." *J. Am. Chem. Soc.* **1993**, *115*, 10531-10537; and (2) Anisfeld, S. T.; Lansbury, P. T. "A Convergent Approach to the Chemical Synthesis of Asparagine-Linked Glycopeptides." *J. Org. Chem.* **1990**, *55*, 5560-5562) with certain modifications: the reported peptide glycosylations involved excess or equimolar amounts of glycosylamine relative to peptide, and their isolated yields (50 - 60%) are reported based on peptide starting material (Cohen-Anisfeld, S. T.; Lansbury, P. T. "A Practical, Convergent Method for Glycopeptide Synthesis." *J. Am. Chem. Soc.* **1993**, *115*, 10531-10537). As is often the case, however, the saccharide here is the more precious material entering glycosylation because its preparation involves multistep, solution phase synthesis in relatively low overall yield compared to that of the peptide. A trial glycosylation of model pentapeptide **40** with pentasaccharide **39** indicates that under the appropriate reaction conditions, an excess of peptide produces a significantly greater yield of coupled product (over 70% based on valuable glycosylamine) [Miller, J. S. *et al., Angew. Chemie Int. Ed.*, 2003, **42**, 431. Subsequent Fmoc (Fmoc = 9-fluorenylmethyloxy-carbonyl) removal with piperidine afforded glycopeptide **41**.

[0175] **Scheme 13.** Exemplary glycopeptide assembly route with a model peptide and glycan.



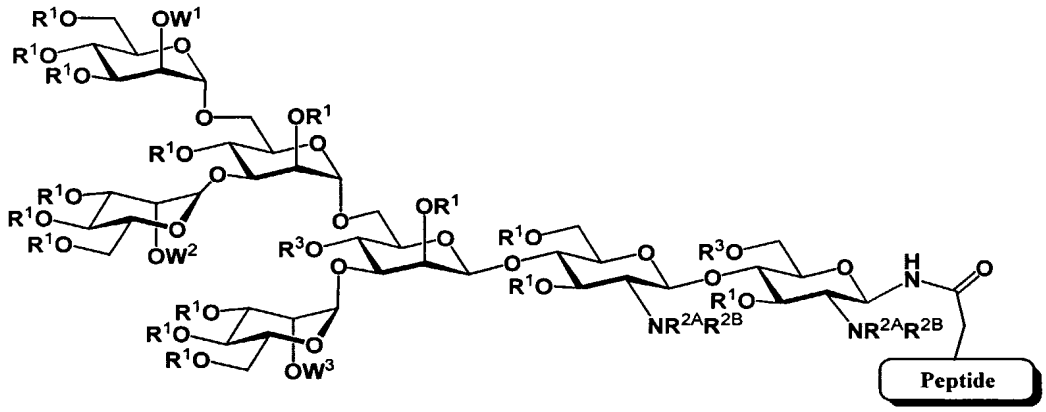
[0176] The final step toward completion of a model glycopeptide involved native chemical ligation (NCL) [See, for example, Dawson, P. E.; Muir, T. W.; Clark-Lewis, I.; Kent, S. B. H. "Synthesis of Proteins by Native Chemical Ligation." *Science* **1994**, *266*, 776-779], as indicated in Scheme 13. *In situ* deprotection of cysteine disulfide **41** and transthioesterification (See, for example, Dawson, P. E.; Churchill, M. J.; Ghadiri, M. R.; Kent, S. B. H. "Modulation of reactivity in native chemical ligation through the use of thiol additives." *J. Am. Chem. Soc.* **1997**, *119*, 4325-4329) of peptide thioester **42** with sodium 2-mercaptoethanesulfonate **(43)** in phosphate-buffered saline (PBS) at neutral pH led to a second thioester exchange with the (now free) cysteine thiol and subsequent rearrangement to give fully unprotected glycopeptide **44**. gp120-derived glycopeptides obtained using the strategy detailed in Scheme 13 will require no additional manipulation other than purification before they can be examined for the generation of antibodies. The synthetic strategy thus requires only four assembly steps starting from free glycans to obtain homogeneous glycopeptides.

[0177] In certain embodiments, the lysine residue is differentially protected with respect to Fmoc removal during peptide synthesis, and remains protected through the peptide glycosylation step (due to its free amine side chain). Suitably protected Lys derivatives have been designed (See, for example, Chhabra, S. R.; Hothi, B.; Evans, D. J.; White, P. D.; Bycroft, B. W.; Chan, W. C. "An appraisal of new variants of Dde amine protecting group for solid phase peptide synthesis." *Tetrahedron Lett.* **1998**, *39*, 1603-1606), and can be deprotected in the presence of *N*-linked saccharides along with the N-terminal Fmoc amine in minutes using hydrazine at room temperature.

[0178] *Peptide Thioester Synthesis*

[0179] Several methods have been developed for peptide thioester synthesis, including the original "Boc chemistry" (Boc = *tert*-butyloxycarbonyl) method (See, for example, (1) Canne, L. E.; Walker, S. M.; Kent, S. B. H. "A General Method for the Synthesis of Thioester Resin Linkers for Use in the Solid-Phase Synthesis of Peptide Alpha-Thioacids." *Tetrahedron Lett.* **1995**, *36*, 1217-1220; and (2) Hojo, H.; Aimoto, S. "Polypeptide Synthesis Using the S-Alkyl Thioester of a Partially Protected Peptide Segment--Synthesis of the DNA- Binding Domain of C-Myb Protein (142-193)-NH2." *Bull. Chem. Soc. Jpn.* **1991**, *64*, 111-117) and several Fmoc-compatible systems (See, for example, (1) Shin, Y.; Winans, K. A.; Backes, B. J.; Kent, S. B. H.; Ellman, J. A.; Bertozzi, C. R. "Fmoc-based synthesis of peptide-(alpha)thioesters: Application to the total chemical synthesis of a glycoprotein by native chemical ligation." *J. Am. Chem. Soc.* **1999**, *121*, 11684-11689; (2) Ingenito, R.; Bianchi, E.; Fattori, D.; Pessi, A. "Solid phase synthesis of peptide C-terminal thioesters by Fmoc/t-Bu chemistry." *J. Am. Chem. Soc.* **1999**, *121*, 11369-11374; (3) Li, X. Q.; Kawakami, T.; Aimoto, S. "Direct preparation of peptide thioesters using an Fmoc solidphase method." *Tetrahedron Lett.* **1998**, *39*, 8669-8672; (4) Clippingdale, A. B.; Barrow, C. J.; Wade, J. D. "Peptide thioester preparation by Fmoc solid phase peptide synthesis for use in native chemical ligation." *J. Pept. Sci.* **2000**, *6*, 225-234; and (5) Bu, X. Z.; Xie, G. Y.; Law, C. W.; Guo, Z. H. "An improved deblocking agent for direct Fmoc solidphase synthesis of peptide thioesters." *Tetrahedron Lett.* **2002**, *43*, 2419-2422). In ceratin embodiments, the model thioester is a C-terminal glycine thioester, which is locally achiral and cannot be epimerized, and is therefore easy to synthesize. Though the desired gp120 thioester contains an epimerization-prone C-terminal histidine (His) residue, such thioesters have been synthesized previously and have in fact been shown to modulate favorably the rate of NCL (See, for example, Hackeng, T. M.; Griffin, J. H.; Dawson, P. E. "Protein synthesis by native chemical ligation: Expanded scope by using straightforward methodology." *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 10068-10073).

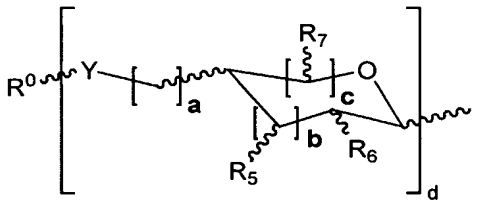
[0180] In another aspect of the present invention, a method of preparing an isolated compound having the structure:



wherein each occurrence of R^1 is independently hydrogen or an oxygen protecting group;

each occurrence of R^{2A} and R^{2B} is independently hydrogen or a nitrogen protecting group;

each occurrence of R^3 is independently hydrogen, a protecting group or a carbohydrate domain comprising a saccharide moiety having the structure:



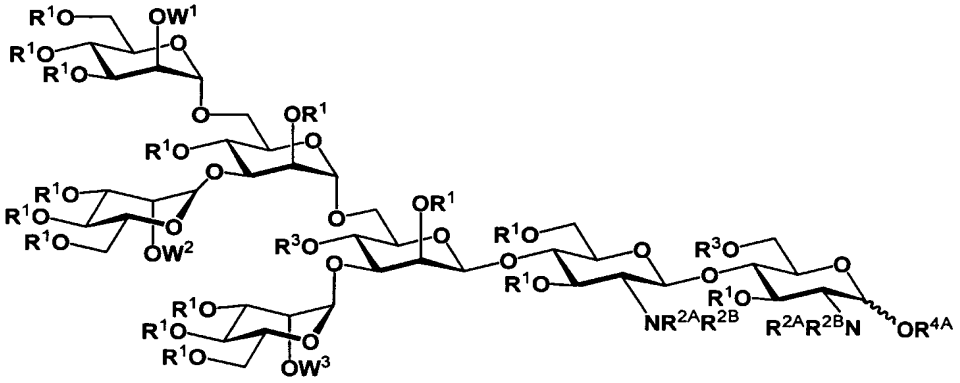
wherein Y is NH or O; wherein a, b and c are each independently 0, 1 or 2; d is an integer from 1-3; with the proviso that the d bracketed structure represents a furanose or pyranose moiety and the sum of b and c is 1 or 2; wherein R^0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein each occurrence of R^5, R^6 and R^7 is independently hydrogen, OH, OR^i, $NR^{ii}R^{iii}$, $NHCOR^i$, F, CH_2OH, CH_2OR^i, or a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of R^i, R^{ii} and R^{iii} is independently hydrogen, a protecting group, a sialic acid moiety, CHO, $COOR^{iv}$, or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group, or R^{ii} and R^{iii}, taken together with the nitrogen atom to which they are attached, form a substituted or unsubstituted heterocyclic or heteroaryl moiety; and wherein each

occurrence of R^{iv} is independently H, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group;

W^1, W^2 and W^3 are independently optionally substituted mannose, galactose or lactosamine moieties;

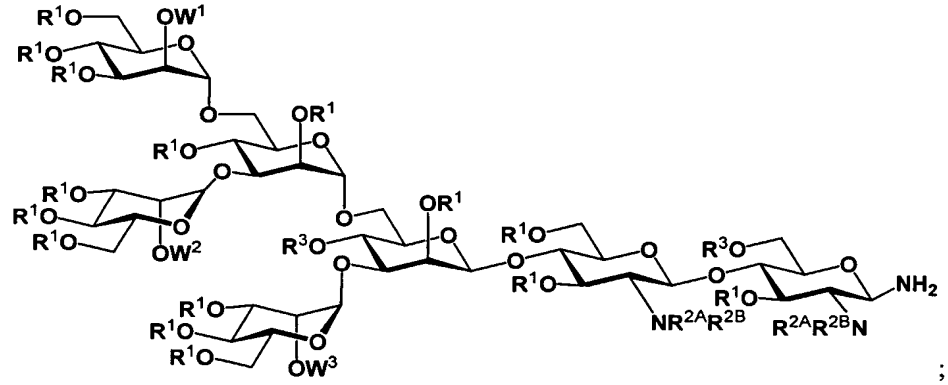
said method comprising steps of:

(a) providing an α-O-protected carbohydrate construct having the structure:



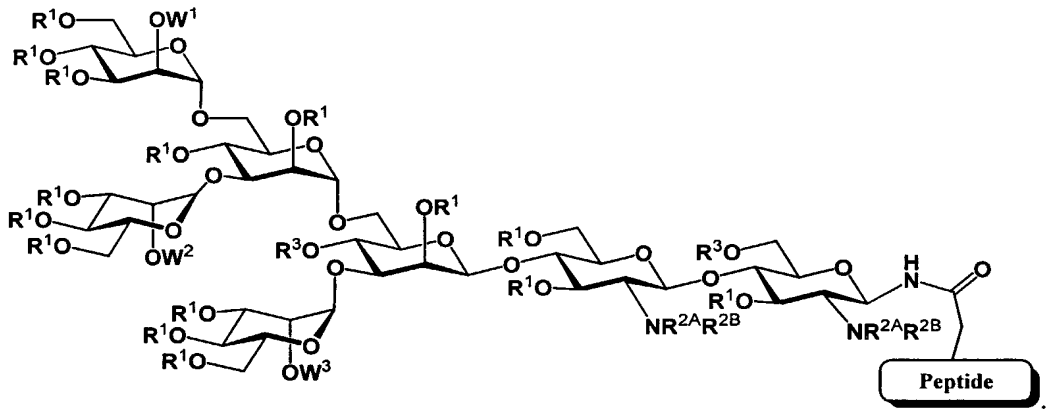
wherein R^{4A} is hydrogen or a suitable oxygen protecting group;

(b) reacting the construct of step (a) under suitable conditions to form a β-amino carbohydrate construct having the structure:

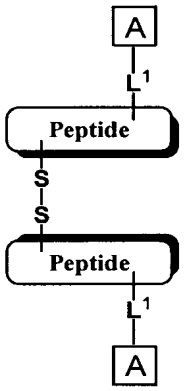


;

(c) reacting said β-amino carbohydrate construct under suitable conditions with a peptide whose structure is either identical or closely related to that of gp120 near an N-glycosylation site and which comprises a $-CH_2CO_2H$ moiety, to form a glycopeptide having the structure:



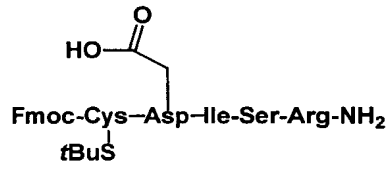
[0181] In certain embodiments, the peptide comprises a cysteine residue and thus, the peptide may be dimerized under suitable oxidization conditions to form the corresponding disulfide dimer. In certain exemplary embodiments, the disulfide dimer has the structure:



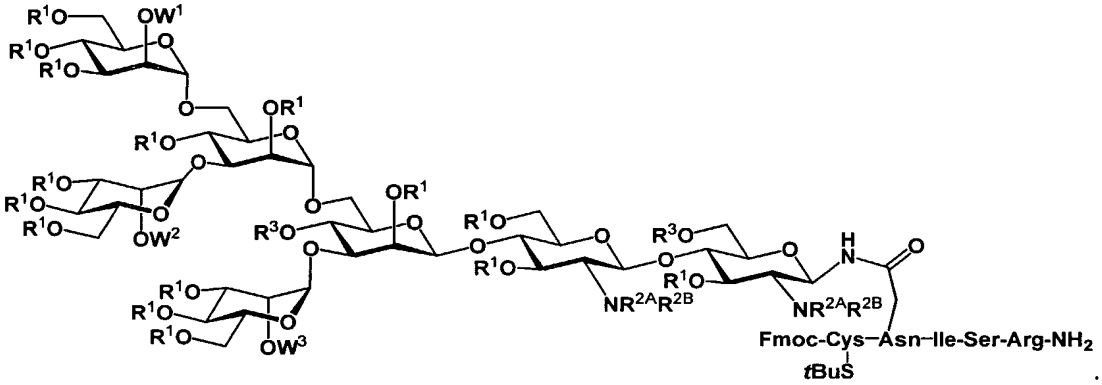
wherein each peptide may be the same or different; each occurrence of L^1 may be the same or different and is as defined above; and each occurrence of A is independently a carbohydrate domain as defined above.

[0182] In certain exemplary embodiments, in the step of reacting the carbohydrate construct of step (a) under suitable conditions to form the β-amino carbohydrate construct, Kochetkov amination conditions are used. In certain exemplary embodiments, in the step of reacting the carbohydrate construct of step (a) under suitable conditions to form the β-amino carbohydrate construct, NH_4HCO_3/H_2O is used. In certain exemplary embodiments, in the β-amino carbohydrate construct of step (b), each occurrence of R^1 and R^3 is hydrogen and each occurrence of $-NR^{2A}R^{2B}$ is –NHAc.

[0183] In certain other exemplary embodiments, in the step of reacting the β-amino carbohydrate construct under suitable conditions with a peptide whose structure is either identical or closely related to that of gp120 near an N-glycosylation site, the reaction conditions comprise HATU and Hünig's base in a suitable solvent. In certain embodiments, the solvent is DMSO. In certain embodiments, the peptide has the following structure:

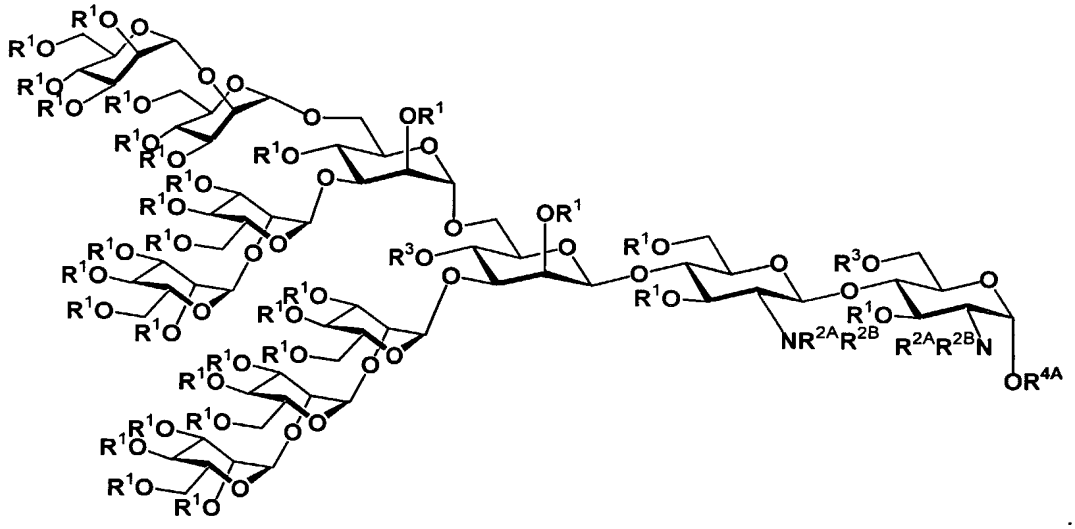


and the glycopeptide of step (c) has the structure:

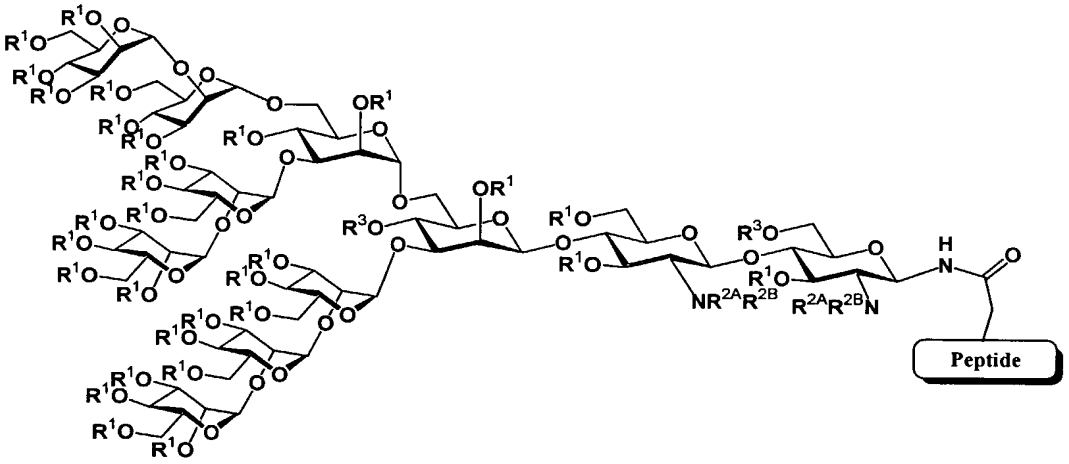


[0184] In certain exemplary embodiments, in the β-amino carbohydrate construct formed in step (b), each occurrence of R^1 and R^3 is hydrogen, each occurrence of $-NR^{2A}R^{2B}$ is –NHAc.

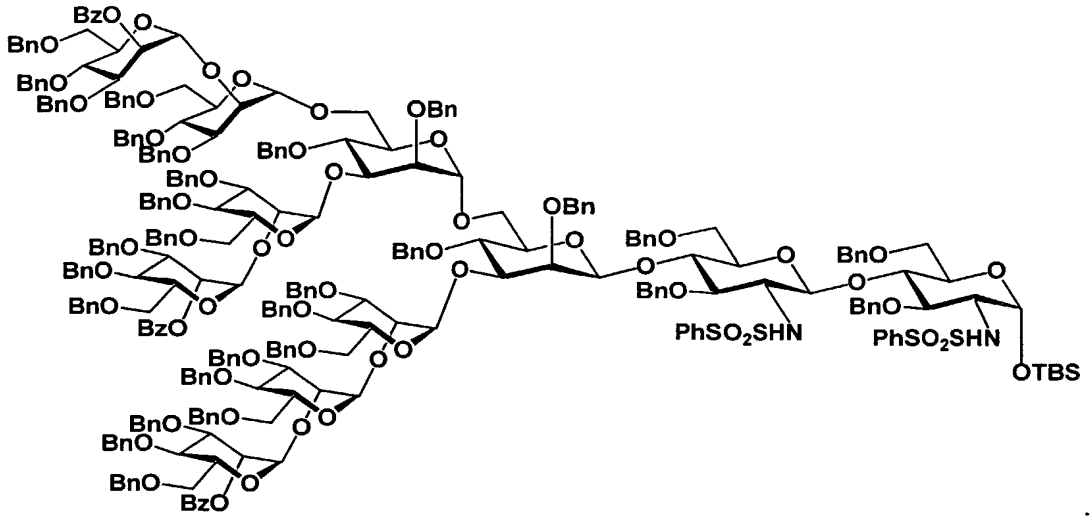
[0185] In certain other exemplary embodiments, the α-O-protected carbohydrate construct of step (a) has the structure:



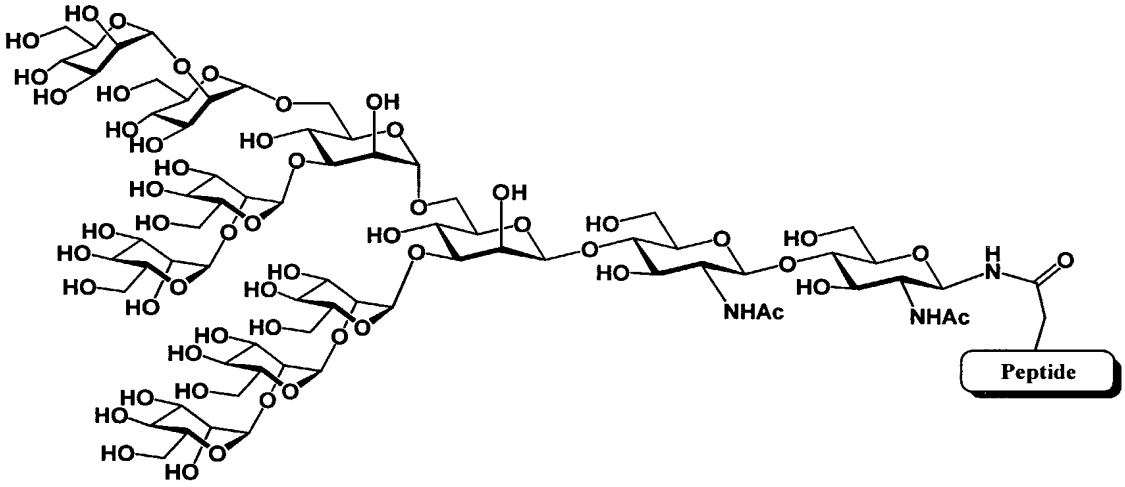
[0186] In certain other exemplary embodiments, the glycopeptide formed in step (c) has the structure:



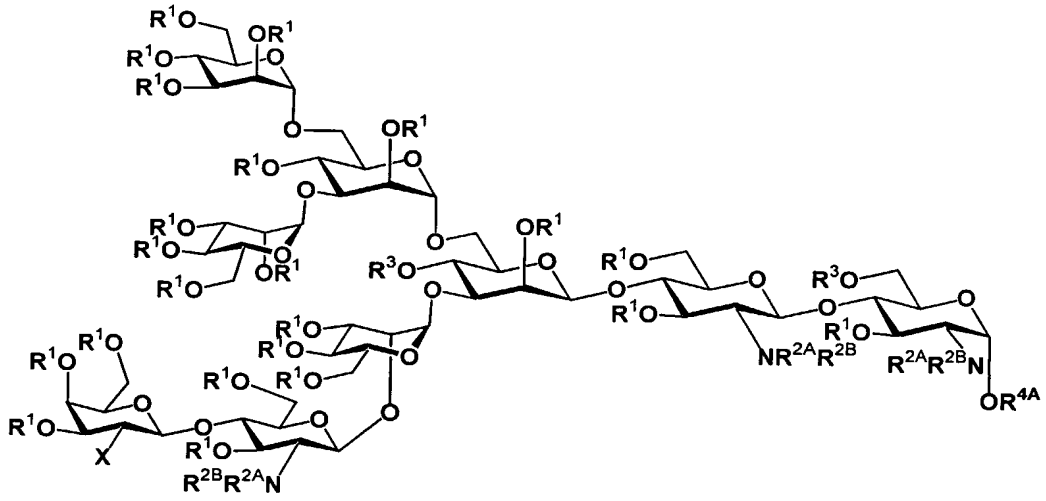
[0187] In certain other exemplary embodiments, the α-O-protected carbohydrate construct of step (a) has the structure:



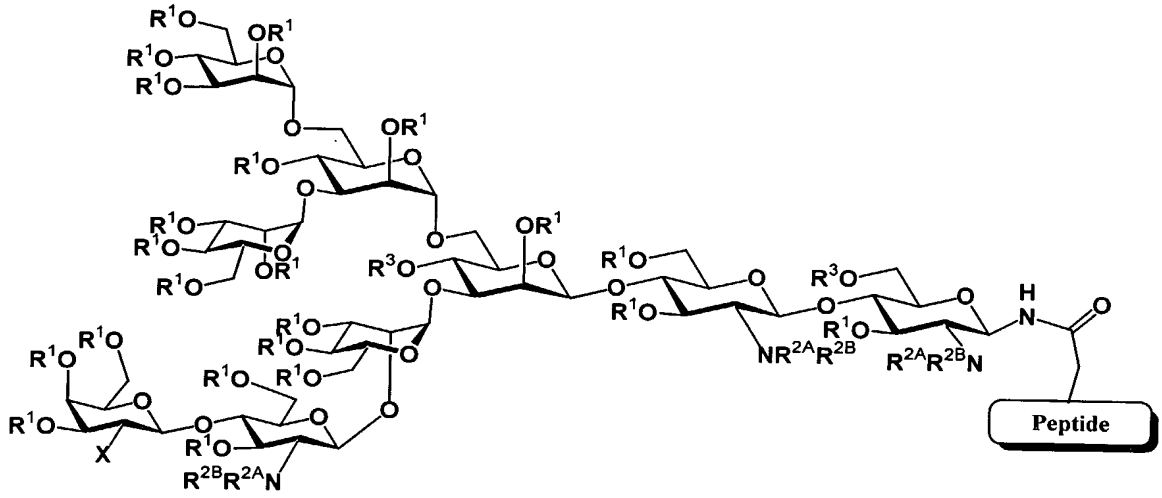
[0188] In certain other exemplary embodiments, the glycopeptide formed in step (c) has the structure:



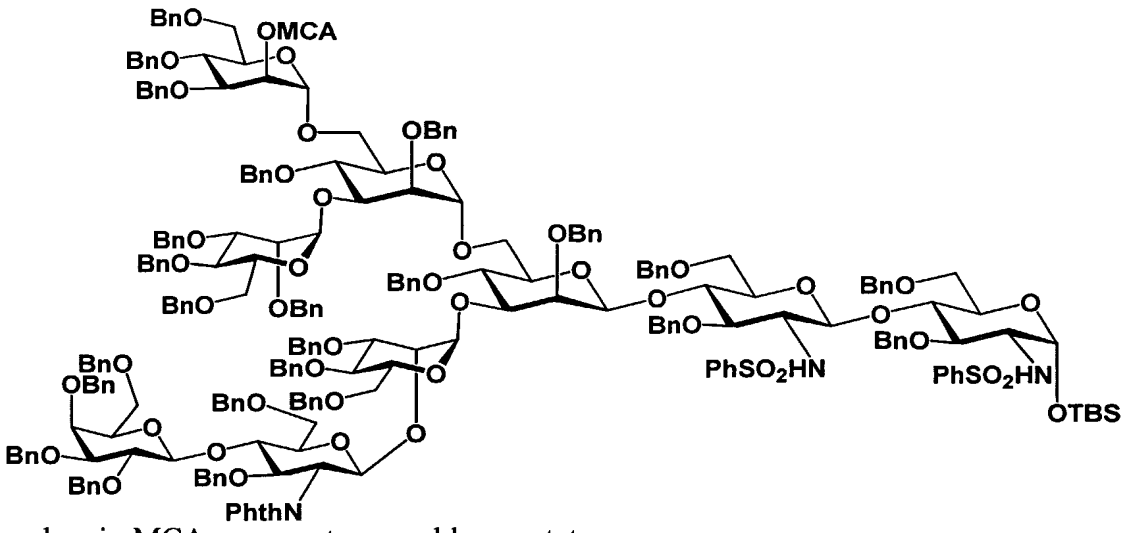
[0189] In certain other exemplary embodiments, the α-O-protected carbohydrate construct of step (a) has the structure:



[0190] In certain other exemplary embodiments, the glycopeptide formed in step (c) has the structure:

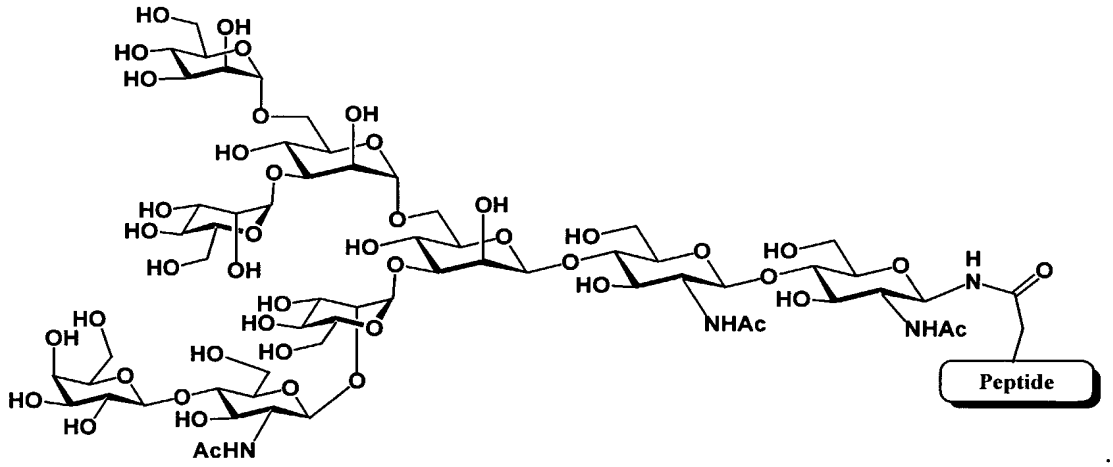


[0191] In certain other exemplary embodiments, the α-O-protected carbohydrate construct of step (a) has the structure:

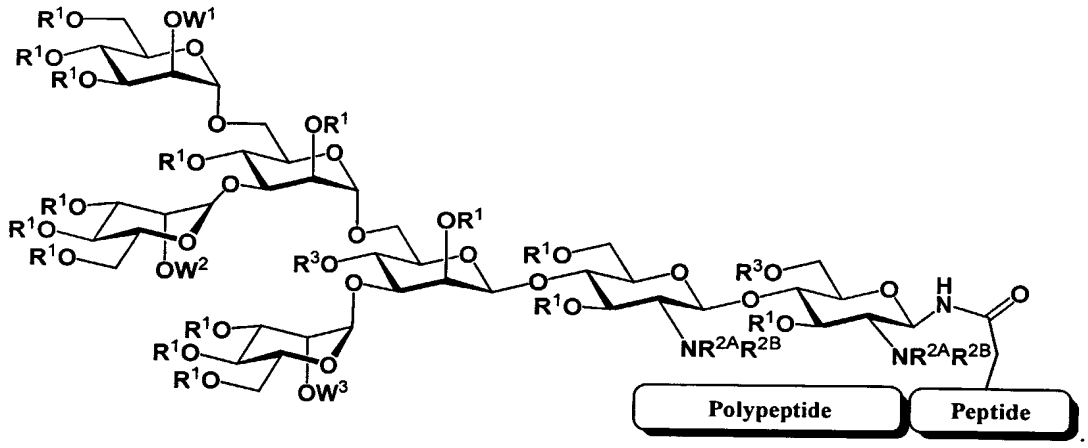


; wherein MCA represent monochloroacetate.

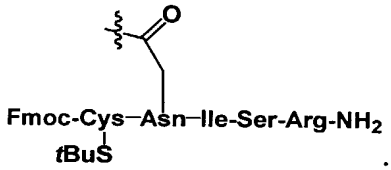
[0192] In certain other exemplary embodiments, the glycopeptide formed in step (c) has the structure:



[0193] In certain other embodiments, the method further comprises a step of subjecting the glycopeptide formed in step (c) to Native Chemical Ligation conditions in the presence of a suitable polypeptide to form a glycopolypeptide having the structure:



[0194] In certain embodiments, the peptide is either identical to or closely related to that of gp120 near an N-glycosylation site and comprises the amino acid sequence: Cys-Asn-Ile-Ser-Arg wherein any one or more of the amino acid residues may bear one or more protecting groups. In certain exemplary embodiments, the carbohydrate construct is attached to an Asparagine residue (Asn) on the peptide via an amide linkage. In certain other exemplary embodiments, the peptide is either identical to or closely related to that of gp120 near an N-glycosylation site and comprises the amino acid sequence:

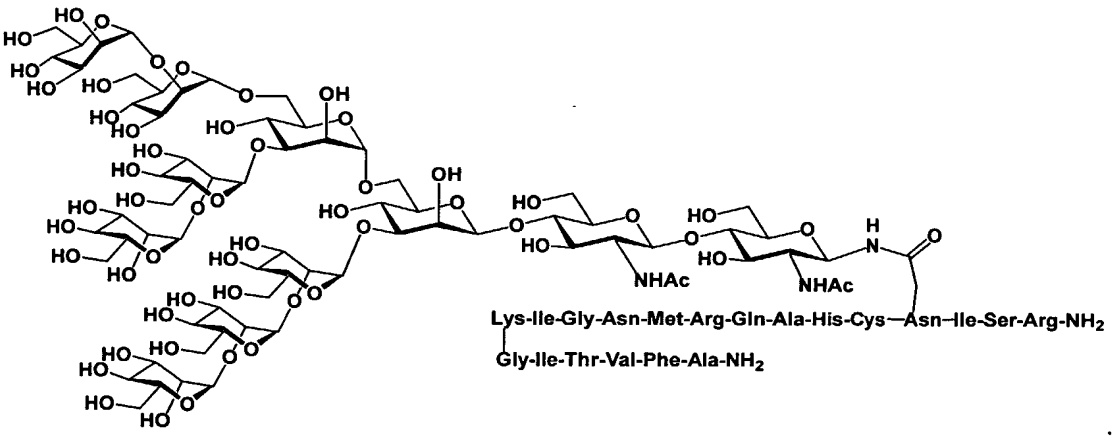


[0195] In certain other embodiments, when the glycopeptide formed in step (c) is further subjected to Native Chemical Ligation, the polypeptide comprises the amino acid sequence: Ala-Phe-Val-Thr-Ile-Gly-Lys-Ile-Gly-Asn-Met-Arg-Gln-Ala-His-Cys-Asn-Ile-Ser-Arg, wherein any one or more of the amino acid residues may bear one or more protecting groups or a moiety suitable for Native Chemical Ligation. In certain embodiments, the polypeptide comprises a moiety suitable for Native Chemical Ligation, wherein the NCL moiety comprises a thioester.

[0196] The synthetic methodology is easily applicable to the generation of significantly longer (or shorter) segments of gp120. Both the peptide to be glycosylated and the thioester utilized for NCL can more closely approach the ~60 residue limit for linear synthesis; the resulting peptide can thus extend entirely to the

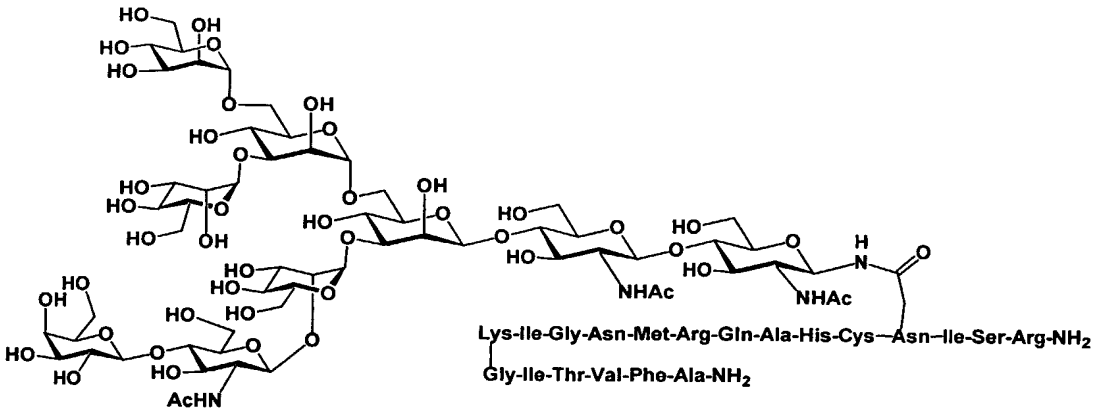
N-terminus of gp120. If the peptide to be glycosylated is extended significantly towards the C-terminus of gp120 the glycosylation yield might suffer due to secondary structure formation of the longer peptide (See, for example, (1) Kent, S. B. H. "Chemical Synthesis of Peptides and Proteins." *Annu. Rev. Biochem.* **1988**, *57*, 957- 989; and (2) Tam, J. P.; Lu, Y. A. "Coupling Difficulty Associated with Interchain Clustering and Phase- Transition in Solid-Phase Peptide-Synthesis." *J. Am. Chem. Soc.* **1995**, *117*, 12058-12063), but reaction conditions involving chaotropic salts have been devised to overcome issues of aggregation (See, for example, Thaler, A.; Seebach, D.; Cardinaux, F. "Lithium Salt Effects in Peptide Synthesis. 2. Improvement of Degree of Resin Swelling and of Efficiency of Coupling in Solid-Phase Synthesis." *Helv. Chim. Acta* **1991**, *74*, 628-643).

[0197] In certain exemplary embodiments, the polypeptide has the structure: Ala-Phe-Val-Thr-Ile-Gly-Lys-Ile-Gly-Asn-Met-Arg-Gln-Ala-His–SR; where R is a functional group suitable for effecting chemical ligation; and the resulting glycopeptide has the structure:



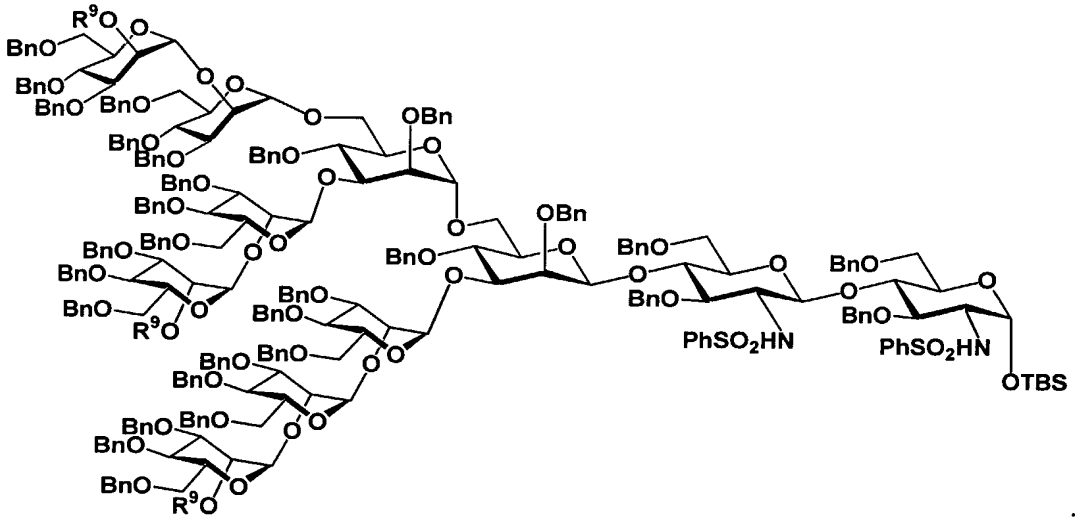
[0198] In certain embodiments, R, in the polypeptide used for native chemical ligation, is $-(CH_2)_2C(=O)NH_2$.

[0199] In certain exemplary embodiments, the polypeptide has the structure: Ala-Phe-Val-Thr-Ile-Gly-Lys-Ile-Gly-Asn-Met-Arg-Gln-Ala-His–SR; where R is a functional group suitable for effecting chemical ligation; and the resulting glycopeptide has the structure:



[0200] In certain embodiments, R, in the polypeptide used for native chemical ligation, is $-(CH_2)_2C(=O)NH_2$.

[0201] In another aspect, the invention provides a method of preparing an α-O-protected carbohydrate construct having the structure:

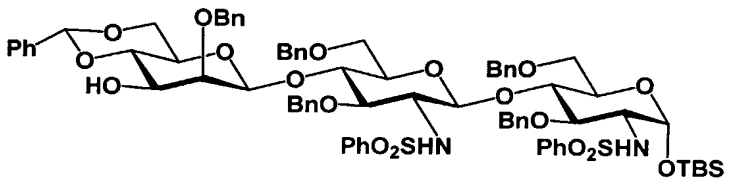


;

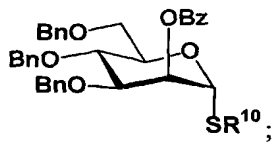
wherein each occurrence of R^9 is independently Bz or Ac;

said method comprising steps of:

(a) coupling a trisaccharide having the structure:

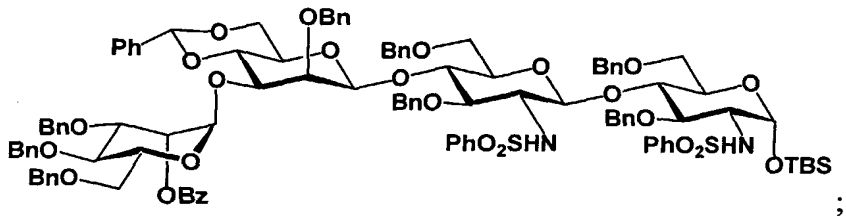


with a monosaccharide having the structure:



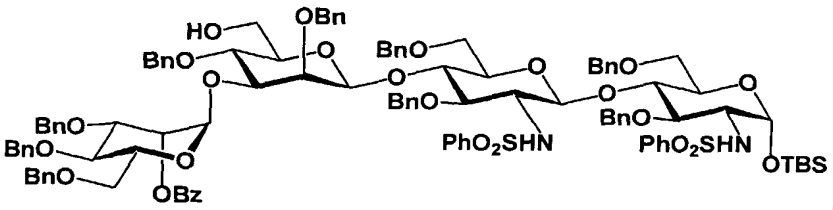
wherein R^{10} is lower alkyl or aryl;

in the presence of an activating agent under suitable conditions to form a protected tetrasaccharide having the structure:



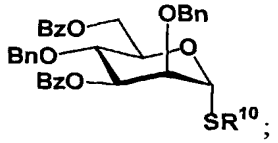
;

(b) partially deprotecting the protected tetrasaccharide formed in step (a) under suitable conditions to form a partially deprotected tetrasaccharide having the structure:



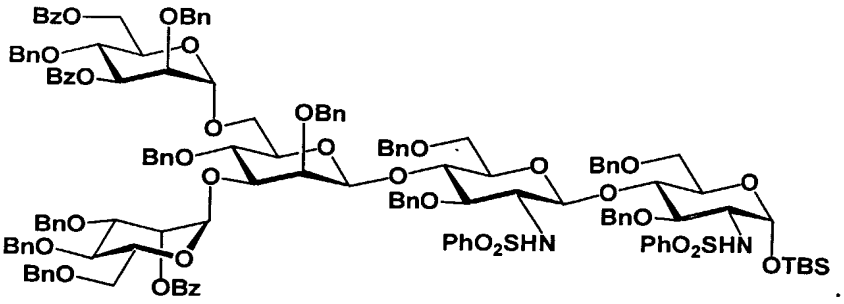
;

(c) coupling the partially deprotected tetrasaccharide formed in step (b) with a monosaccharide having the structure:



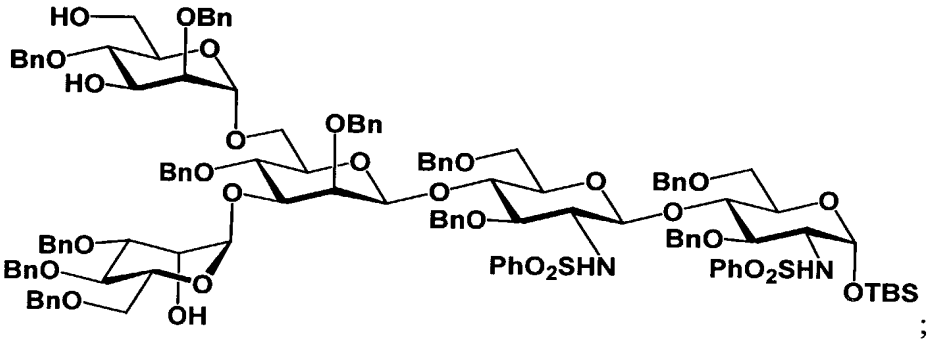
wherein R^{10} is lower alkyl or aryl;

in the presence of an activating agent under suitable conditions to form a protected pentasaccharide having the structure:



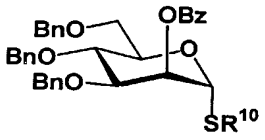
;

(d) partially deprotecting the pentasaccharide formed in step (c) under suitable conditions to form a partially deprotected pentasaccharide having the structure:



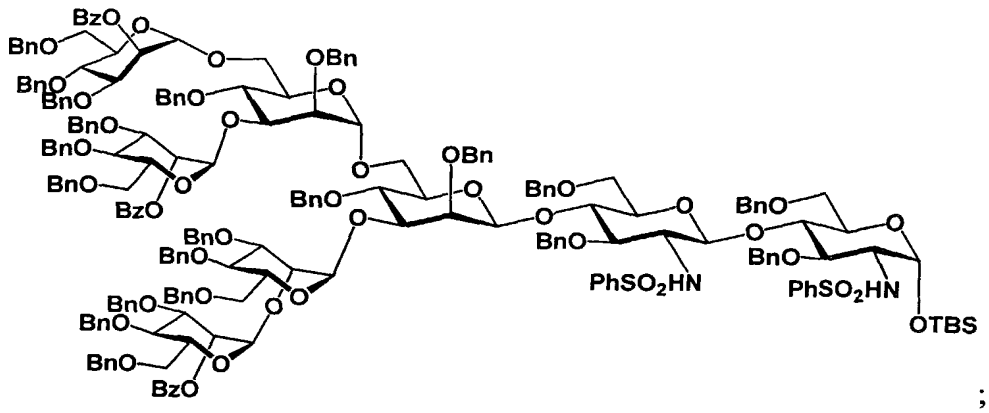
;

(e) coupling the partially deprotected pentasaccharide formed in step (d) with a monosaccharide having the structure:



wherein R^{10} is lower alkyl or aryl;

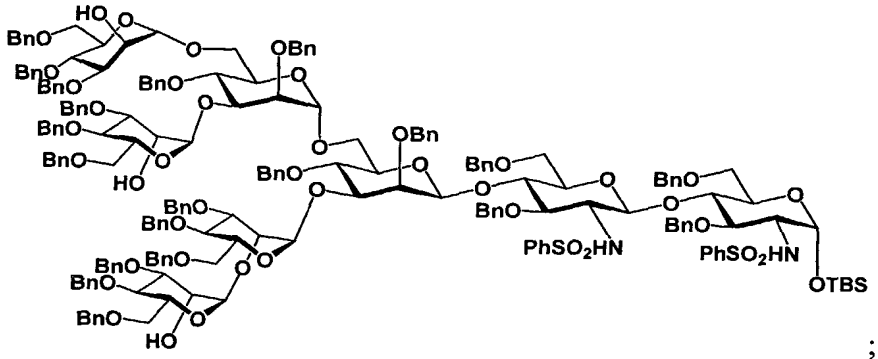
in the presence of an activating agent under suitable conditions to form an octasaccharide having the structure:



;

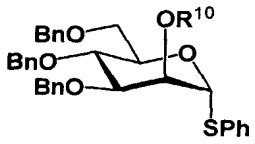
and

(f) partially deprotecting the octasaccharide formed in step (e) under suitable conditions to form a partially deprotected octasaccharide having the structure:



; and

(g) coupling the partially deprotected octasaccharide formed in step (f) with a monosaccharide having the structure:

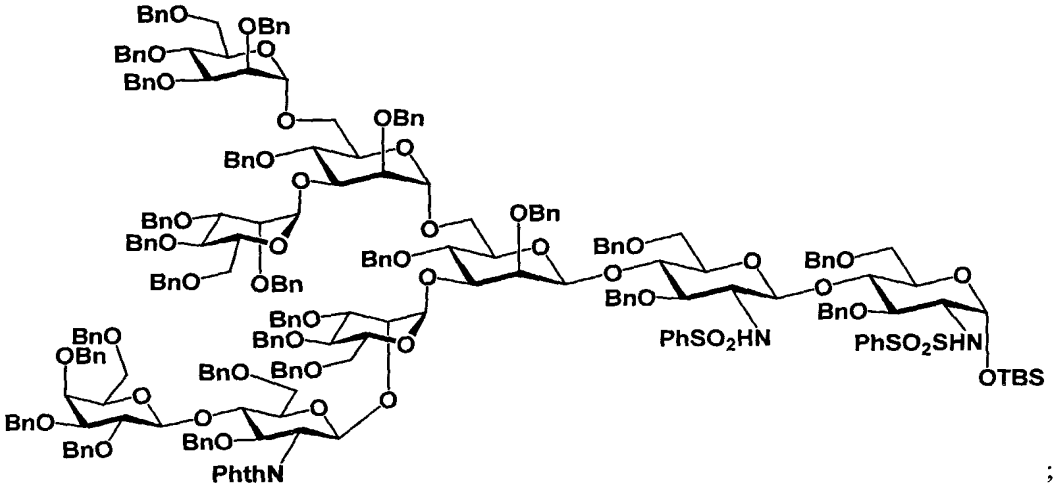


in the presence of an activating agent under suitable conditions to the α-O-protected carbohydrate construct.

[0202] In certain exemplary embodiments, the activating agent used in steps (a), (c), (e) and (g) comprises $(BrC_6H_4)_3NSbCl_6$. In certain other exemplary embodiments, in the step of partially deprotecting the protected tetrasaccharide (step (b)), the protected tetrasaccharide formed in step (a) is subjected to reductive reaction conditions comprising Bu_2BOTf, BH_3. In certain other exemplary embodiments, in the step of partially deprotecting the protected pentasaccharide

(step (d)), the protected pentasaccharide formed in step (c) is subjected to reaction conditions comprising NaOMe. In certain other exemplary embodiments, in the step of partially deprotecting the protected octasaccharide (step (f)), the protected octasaccharide formed in step (e) is subjected to reaction conditions comprising NaOMe.

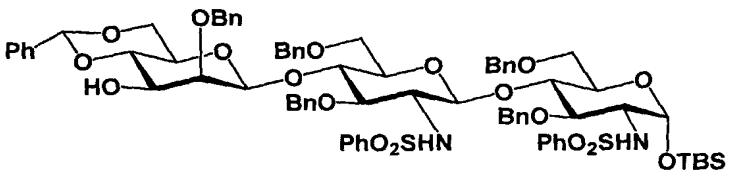
[0203] In another aspect, the invention provides a method of preparing an α-O-protected carbohydrate construct having the structure:



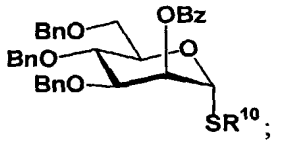
;

said method comprising steps of:

(a) coupling a trisaccharide having the structure:



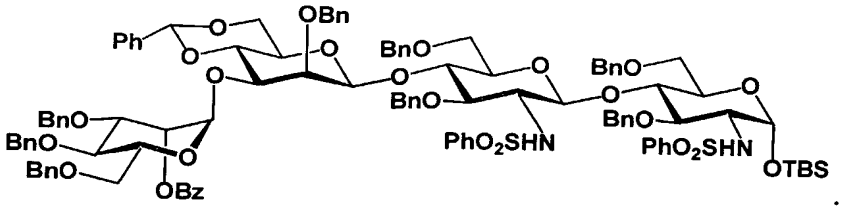
with a monosaccharide having the structure:



;

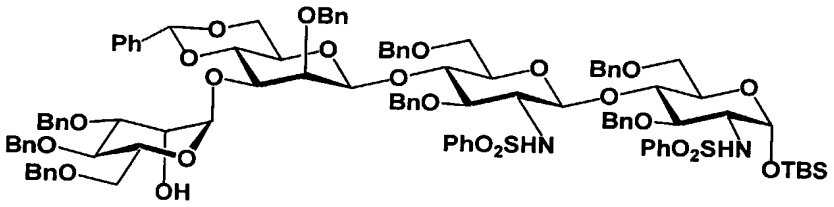
wherein R^{10} is lower alkyl or aryl;

in the presence of an activating agent under suitable conditions to form a protected tetrasaccharide having the structure:

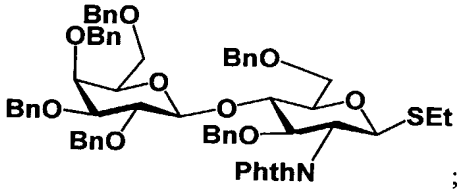


;

(b) partially deprotecting the protected tetrasaccharide formed in step (a) under suitable conditions to form a partially deprotected tetrasaccharide having the structure:

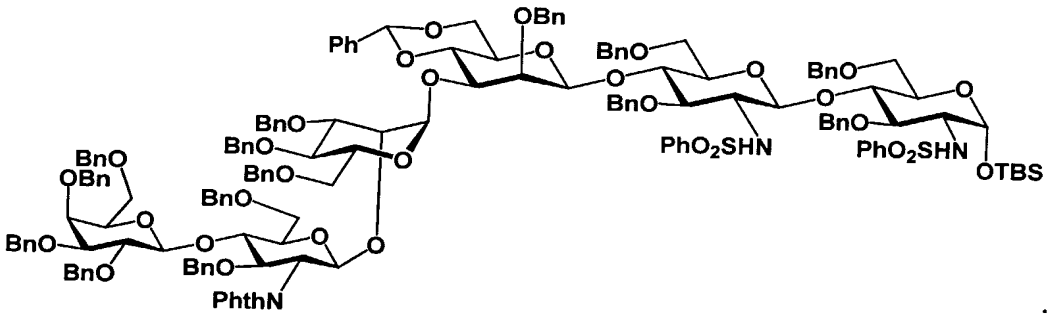


(c) coupling the partially deprotected tetrasaccharide formed in step (b) with an ethylthioglycoside having the structure:



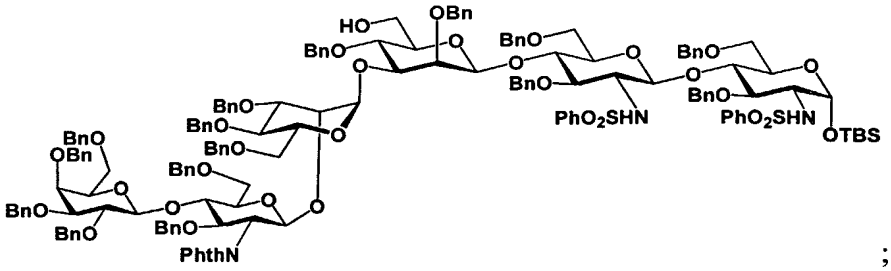
;

under suitable conditions to form a protected hexasaccharide having the structure:



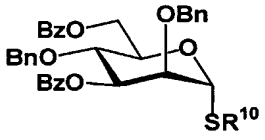
;

(d) partially deprotecting the hexasaccharide formed in step (c) under suitable conditions to form a partially deprotected hexasaccharide having the structure:



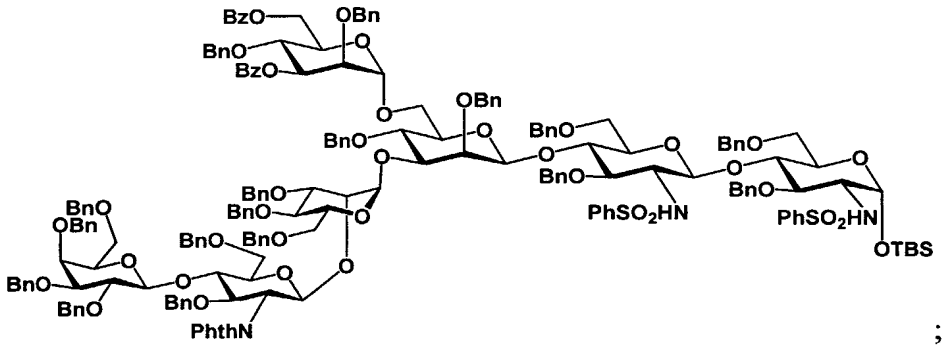
;

(e) coupling the partially deprotected hexasaccharide formed in step (d) with a monosaccharide having the structure:



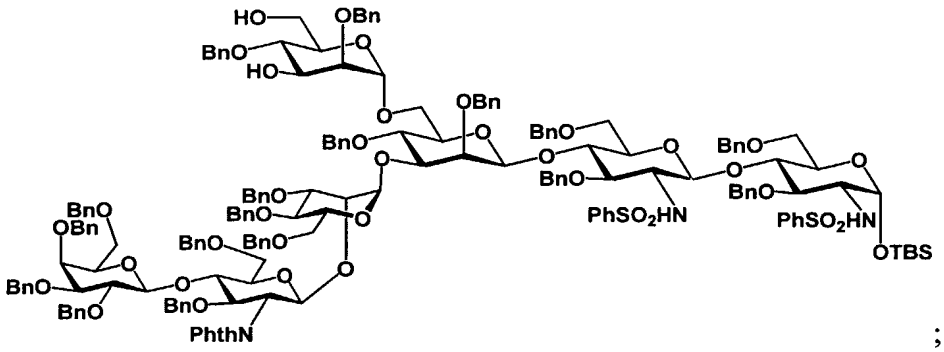
wherein R^{10} is lower alkyl or aryl;

in the presence of an activating agent under suitable conditions to form an heptasaccharide having the structure:



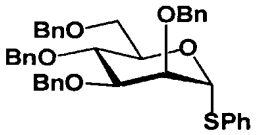
; and

(f) partially deprotecting the heptasaccharide formed in step (e) under suitable conditions to form a partially deprotected heptasaccharide having the structure:



; and

(g) coupling the partially deprotected heptasaccharide formed in step (f) with a monosaccharide having the structure:



in the presence of an activating agent under suitable conditions to the α-O-protected carbohydrate construct.

[0204] In certain embodiments, R^{10} is ethyl or phenyl.

[0205] In certain exemplary embodiments, the activating agent used in steps (a), (e) and (g) comprises $(BrC_6H_4)_3NSbCl_6$. In certain other exemplary embodiments, in the step of partially deprotecting the protected hexasaccharide (step (d)), the protected hexasaccharide formed in step (c) is subjected to reductive reaction conditions comprising Bu_2BOTf, BH_3. In certain other exemplary embodiments, in the step of partially deprotecting the protected tetrasaccharide (step (b)), the protected tetrasaccharide formed in step (a) is subjected to reaction conditions comprising NaOMe. In certain other exemplary embodiments, in the step of partially deprotecting the protected heptasaccharide (step (f)), the protected heptasaccharide formed in step (e) is subjected to reaction conditions comprising NaOMe.

[0206] It will be appreciated that for each of the methods as detailed herein, the full arsenal of protecting groups known in the art of organic synthesis can be utilized, for example, as set forth in "Activating Agents and Protecting Groups: Handbook of Reagents for Organic Synthesis" Roush, W.R. and Pearson, A.J., Eds., John Wiley & Sons: 1999; and "Protective Groups in Organic Synthesis" Greene, T.W. and Wuts, P.G., John Wiley & Sons, New York: 1999, the entire contents of which are hereby incorporated by reference. In but a few examples, suitable protecting groups utilized herein include, but are not limited to, Bn (benzyl), TIPS (triisopropylsilyl), and Ac (acetate). In a certain exemplary embodiments of the present invention, coupling of glycoside moieties are effected under MeOTf promotion, as described herein. It will be appreciated by one of ordinary skill in the art however, that a variety of conditions known in the art of organic synthesis can be utilized to effect coupling of glycoside moieties.

[0207] The skilled practitioner will know how to adapt the synthetic methods detailed in the present invention to access a variety of other multi-branched gp120 glycans and constucts thereof.

[0208] In certain other exemplary embodiments, the construct should be so functionalized as to anticipate the need for its conjugation to an immunogenic carrier (*e.g.*, protein or lipid) in anticipation of the need to stimulate an immune response. As discussed above, such constructs may be used to generate antibodies for use in HIV vaccine. The present invention provides improvements in total synthesis and HIV therapy. For example, as discussed extensively herein, the present invention provides novel glycopeptide synthetic methodology that allows access to complex glycans linked to various backbones.

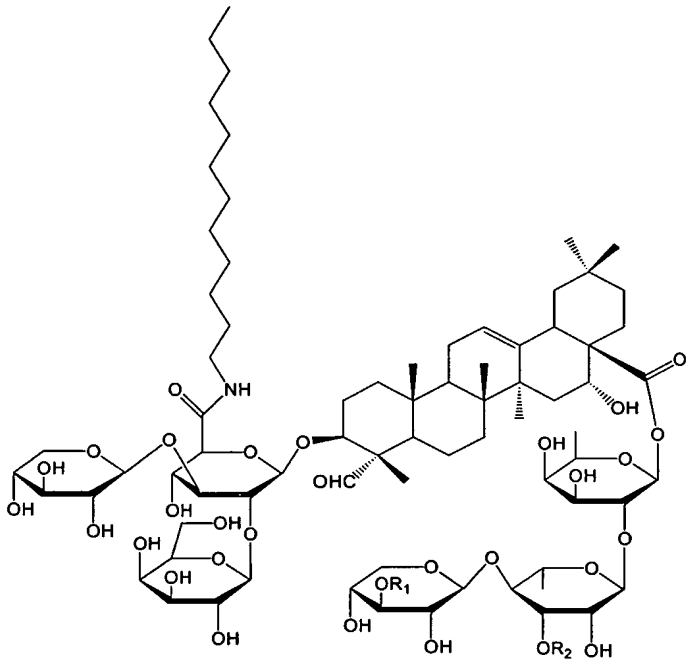
[0209] As discussed above, in one embodiment of the present invention, the inventive compounds can be conjugated either directly or through a crosslinker to an appropriate carrier (*e.g.*, KLH) to generate a synthetic tumor antigen. Methods of conjugation are well known in the art. For example, a conjugation strategy may be employed that involves a reductive coupling of an aldehyde (CHO) functionality on the antigenic compound, with the intended protein carrier, or lipid, presumably at the ε-amino acid residues of exposed lysines. (M.A. Bernstein; L.D. Hall, *Carbohydr. Res.* **1980**, *78*, C1; R.V. Lemieux *Chem. Soc. Rev.* **1978**, *7*, 423). Thus, in another aspect, the present invention provides synthetic constructs, whereby novel antigenic structures, as described herein, are conjugated to immunogenic carriers (*e.g.*, proteins, peptides or lipids).

[0210] In summary, there is provided a method for gp120 glycan synthesis that is easily modified to incorporate higher degrees of carbohydrate branching. In addition, the inventive synthetic method allows the incorporation of synthetic glycans into relatively long gp120 peptides using a fast, high-yielding strategy that remains synthetically flexible. Accordingly, the glycopeptide structures may be optimized based on their abilities to generate antibodies for use in an HIV vaccine.

[0211] **3) Compositions**

[0212] In another aspect, the present invention provides compositions comprising any one or more of the inventive gp120 glycans and/or constructs thereof.

[0213] In certain embodiments, the inventive compositions may comprise an adjuvant. In certain embodiments, the adjuvant is a saponin adjuvant (see, *e.g.*, Marciani *et al.*, *Vaccine*, **2000**, *18*, 3141, US Patent No.: 6,080,725 and 5,977,081, the entire contents of which are hereby incorporated by reference). One example of a preferred saponin adjuvant includes, but is not limited to, GPI-0100, (Galenica Pharmaceuticals, Inc., Frederick, MD) which is a semi-synthetic adjuvant derived by modifying selected natural saponins.



GPI-0100

[0214] Saponins isolated from *Quillaja soponaria Molina* contain two acyl moieties, a normonoterpene carboxylic acid and a normonoterpene carboxylic acid glycoside, which are linked linearly to a fucosyl residue attached at position C-28. It has been hypothesized that these lipophilic acyl groups may be responsible for these saponins' toxicity and their ability to stimulate cytotoxic T cells against exogenous antigens. The linkage between the fucosyl residue and the acyl group is unstable and hydrolyzes under mild conditions (pH≥6) with concomittant loss of saponins capability to stimulate cell-mediated immune response. Unlike their saponin precursors, GPI-0100 adjuvants comprise a stable non-toxic lipophilic moiety in the saponin's glucuronic residue. Methods for preparing these semi-synthetic adjuvants

are well-known in the art. For example, GPI-0100 adjuvants may be prepared by hydrolizing quillaja saponins (which are commercially available) under basic conditions to yield the corresponding deacylated product. The deacylated intermediate may then be reacted with a suitable amine reagent using standard carboxylic acid moiety activation methodology to give the desired compounds. A wide variety of procedures are effective for extrating saponin compounds. They are generalized as follows: (i) defatting of the organic matter with a hydrophobic organic solvent such as petroleum ether; (ii) extraction with a suitable alcohol (*e.g.*, methanol or ethanol) or alcohol-water mixture; (iii) evaporation of the carinol solvent; and (iv) partitioning of the dried alcohol extract between water and n-butanol saturated with water, followed by precipitation of the crude saponins from the n-butanol/water with a suitable organic solvent (*e.g.*, diethyl ether). Purification of the saponin extract may require multiple separation steps. For example, preliminary fractionation may be carried out using conventional open column chromatography or flash chromatography on silica gel, in combination with a more sophisticated chromatographic technique such as High Pressure Liquid Chromatography (HPLC), droplet counter-current liquid chromatography (DCCC) or centrifugal Liquid Chromatography (RLCC). The integration of these techniques with preparative TLC typically affords separated and purified saponins.

[0215] In certain other preferred embodiments, the adjuvant is bacteria or liposomes. In certain examples, the adjuvant includes but is not limited to, *Salmonella minnesota* cells, bacille Calmette-Guerin or QS21.

[0216] As described above, the present invention provides compounds and synthetic methodologies useful in the development of novel therapeutic agents, particularly for fully synthetic HIV vaccines and/or therapeutics. In general, the compounds (e.g., gp120 glycans, glycopeptides thereof and other constructs thereof) prepared as disclosed herein can be conjugated to a protein carrier or a lipid to generate useful glycoconjugates for the treatment and/or prevention of HIV in a subject suffering therefrom. In addition, glycoconjugates prepared by processes disclosed herein are useful in adjuvant therapies as vaccines capable of inducing a potent and broad neutralizing antibody response. Such adjuvant therapies may reduce the rate of progression of HIV and/or prevent the onset of HIV.

[0217] Thus, the present invention provides pharmaceutical compositions for treating HIV, and for preventing the onset or progression of HIV, comprising any of the compounds of the present invention disclosed herein, as an active ingredient, optionally, though typically in combination with a pharmaceutically acceptable carrier. The pharmaceutical compositions of the present invention may further comprise other therapeutically active ingredients (*e.g.*, anti-HIV and/or palliative agents). For purposes of the invention, the term *"Palliative"* refers to treatment that is focused on the relief of symptoms of a disease and/or side effects of a therapeutic regimen, but is not curative. For example, palliative treatment encompasses painkillers, antinausea medications and anti-sickness drugs.

[0218] The inventive compositions include those suitable for oral, rectal, topical (including transdermal devices, aerosols, creams, ointments, lotions and dusting powders), parenteral (including subcutaneous, intramuscular, and intravenous), ocular (opthalmic), pulmonary (nasal or buccal inhalation) or nasal administration. Although the most suitable route in any given case will depend largely on the nature and severity of the condition being treated and on the nature of the active ingredient. They may be conveniently presented in unit dosage form and prepared by any of the methods well known in the art of pharmacy. In certain embodiments, the compositions are suitable for parenteral administration. In certain exemplary embodiments, the compositions are suitable for intravenous administration.

[0219] In preparing oral dosage forms, any of the unusual pharmaceutical media may be used, such as water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents, and the like in the case of oral liquid preparations (*e.g.*, suspensions, elixers and solutions); or carriers such as starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, disinterating agents, etc., in the case of oral solid preparations are preferred over liquid oral preparations such as powders, capsules and tablets. If desired, capsules may be coated by standard aqueous or non-aqueous techniques. In addition to the dosage forms described above, the compounds of the invention may be administered by controlled release means and devices.

[0220] Pharmaceutical compositions of the present invention suitable for oral administration may be prepared as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient in powder or granular form or as a solution or suspension in an aqueous or nonaqueous liquid or in an oil-in-water or water-in-oil emulsion. Such compositions may be prepared by any of the methods known in the art of pharmacy. In general, compositions are prepared by uniformly and intimately admixing the active ingredient with liquid carriers, finely divided solid carriers, or both and then, if necessary, shaping the product into the desired form. For example, a tablet may be prepared by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granule optionally mixed with a binder, lubricant, inert diluent or surface active or dispersing agent. Molded tablets may be made by molding in a suitable machine, a mixture of the powdered compound moistened with an inert liquid diluent.

[0221] **4) Pharmaceutical Uses and Methods of Treatment**

[0222] *Pharmaceutical Uses*

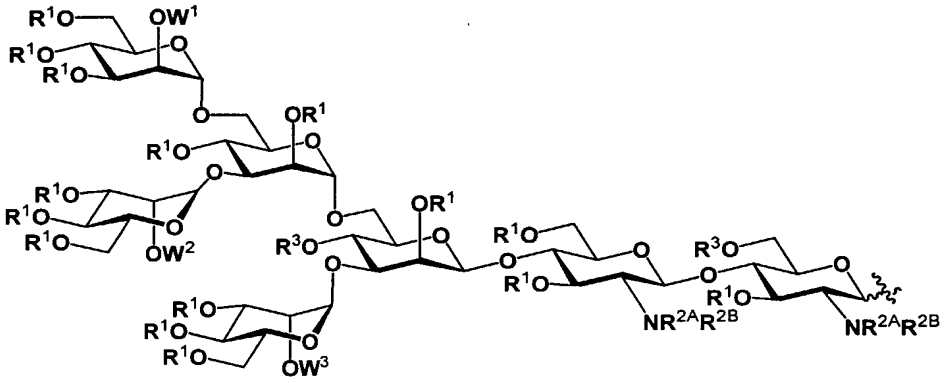
[0223] In one aspect, the present invention provides gp120 glycans and constructs thereof for use as active pharmaceutical agent useful for preventing or reducing the rate of infection with HIV in subjects.

[0224] In another aspect, the inventive gp120 glycans and constructs thereof may be used to raise antibodies specific to HIV virus. In another aspect, the invention provides an antibody which is specific to one or more gp120 glycans and/or constructs thereof described herein.

[0225] Accordingly, in one aspect of the invention, there is provided an antibody or antibody fragment which is specific to one or more of the inventive gp120 glycans and/or glycoconjugates thereof described herein, said antibody being a purified polyclonal antibody or a monoclonal antibody. As used herein, the term "antibody fragment" is generally intended to mean any antibody fragment having conserved the specificity of the antibody of origin, and in particular fragments of the Fab and F(ab^{1}) type. Unless otherwise indicated, the term "antibody" also subsequently denotes antibody fragments when appropriate. The expression

"antibody which binds specifically to gp120 antigen" or "antibody which is specific to gp120 antigen" is intended to denote, an antibody which binds to one or more gp120 glycans described herein, with high specificity. For example, in certain embodiments, the product which is bound to the antibody consists of at least 80% and preferably of at least 90%, of said gp120 antigen.

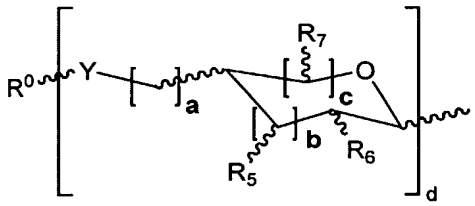
[0226] Thus, in one aspect, the invention provides an antibody or antibody fragment which is specific to any one of the inventive antigens (independently of the others) present on a multi-antigenic construct comprising one or more carbohydrate domains having the structure:



wherein each occurrence of R^1 is independently hydrogen or an oxygen protecting group;

each occurrence of R^{2A} and R^{2B} is independently hydrogen or a nitrogen protecting group;

each occurrence of R^3 is independently hydrogen, a protecting group or a carbohydrate domain comprising a saccharide moiety having the structure:



wherein Y is NH or O; wherein a, b and c are each independently 0, 1 or 2; d is an integer from 1-3; with the proviso that the d bracketed structure represents a furanose or pyranose moiety and the sum of b and c is 1 or 2; wherein R^0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein

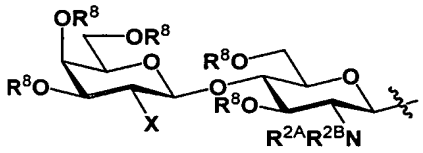
each occurrence of R^5, R^6 and R^7 is independently hydrogen, OH, OR^i, $NR^{ii}R^{iii}$, $NHCOR^i$, F, CH_2OH, CH_2OR^i, or a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of R^i, R^{ii} and R^{iii} is independently hydrogen, a protecting group, a sialic acid moiety, CHO, $COOR^{iv}$, or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group, or R^{ii} and R^{iii}, taken together with the nitrogen atom to which they are attached, form a substituted or unsubstituted heterocyclic or heteroaryl moiety; and wherein each occurrence of R^{iv} is independently H, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group;

W^1, W^2 and W^3 are independently optionally substituted mannose, galactose or lactosamine moieties;

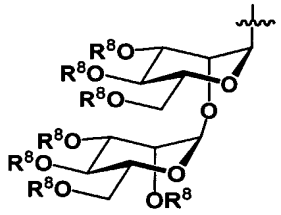
wherein each carbohydrate domain is independently covalently bound to a linker system, said linker system being a peptide or non-peptide nature; and wherein the linker system may be cyclic or acyclic; and

and wherein said antibody is a purified polyclonal antibody or a monoclonal antibody. In certain embodiments, the antibody is a monoclonal antibody.

[0227] In certain embodiments, W^3 is R^1, R^3, as defined above, or a moiety having the structure:

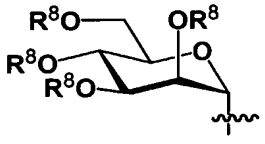


or



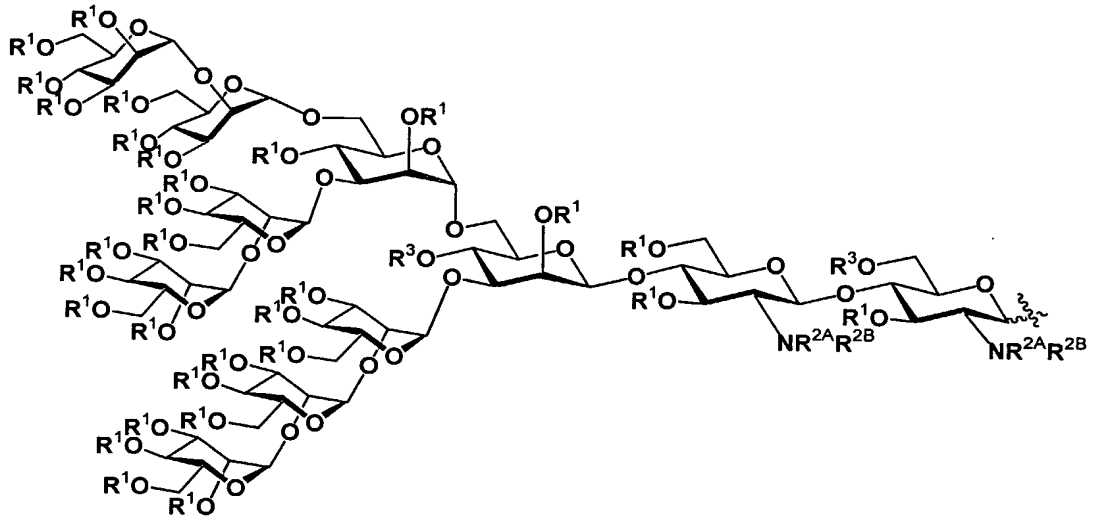
wherein X is $-OR^1$ or $-NR^{2A}R^{2B}$; and each occurrence of R^8 is independently R^1 or a sialic acid moiety.

[0228] In certain embodiments, W^1 and W^2 are independently R^1, R^3 or a moiety having the structure:

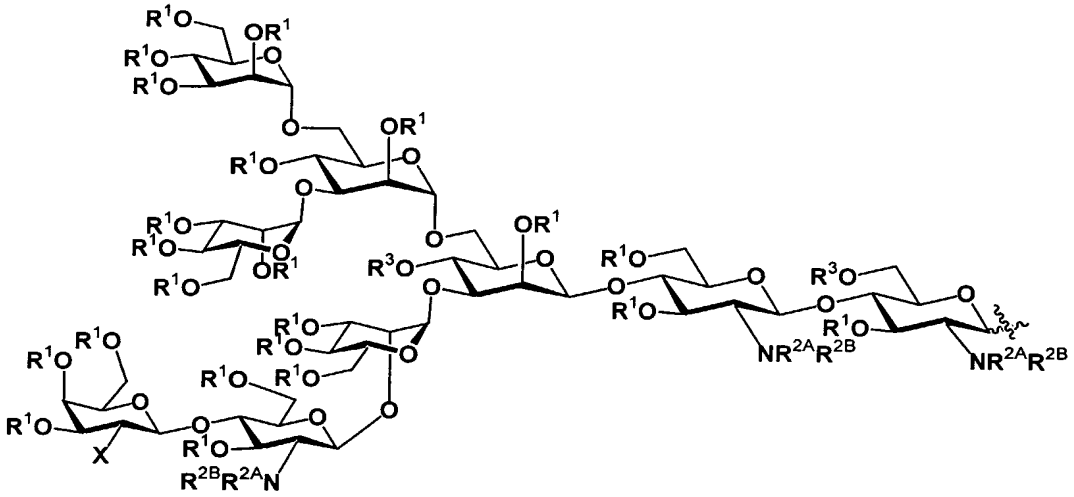


wherein each occurrence of R^8 is independently R^1 or a sialic acid moiety.

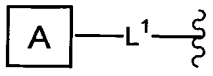
[0229] In certain other embodiments, the antigen comprises a carbohydrate domain having the structure:



In certain other embodiments, the antigen comprises a carbohydrate domain having the structure:

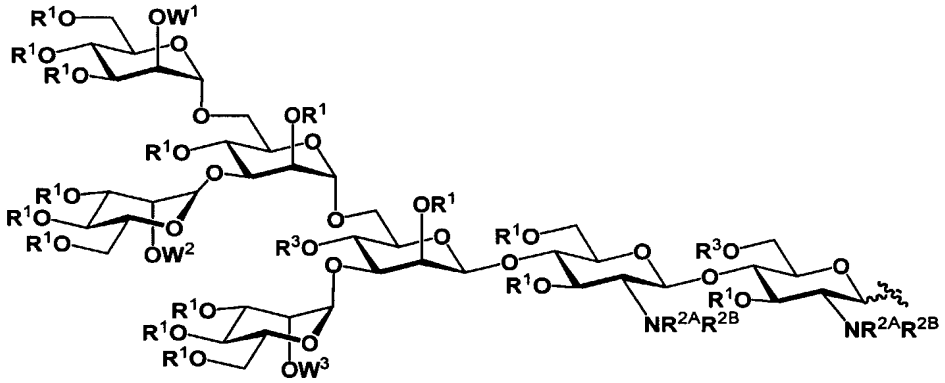


[0230] In certain embodiments, the invention provides an antibody or antibody fragment which is specific to any one or more of the inventive antigens present on a multi-antigenic construct comprising a cyclic or acyclic peptidic or non-peptidic backbone made up of two or more structural units, wherein one or more of said structural units is/are independently substituted with a glycosidic moiety having the structure:

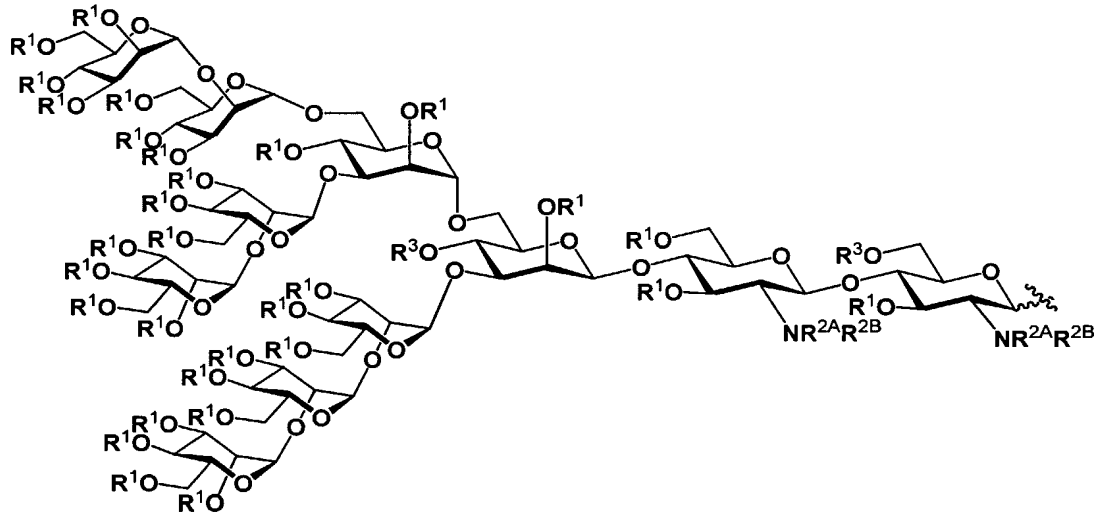


wherein each occurrence of L^1 is independently a substituted or unsubstituted, linear or branched, cyclic or acyclic, saturated or unsaturated aliphatic or heteroaliphatic moiety; and

each occurrence of A is independently a carbohydrate domain of formula:

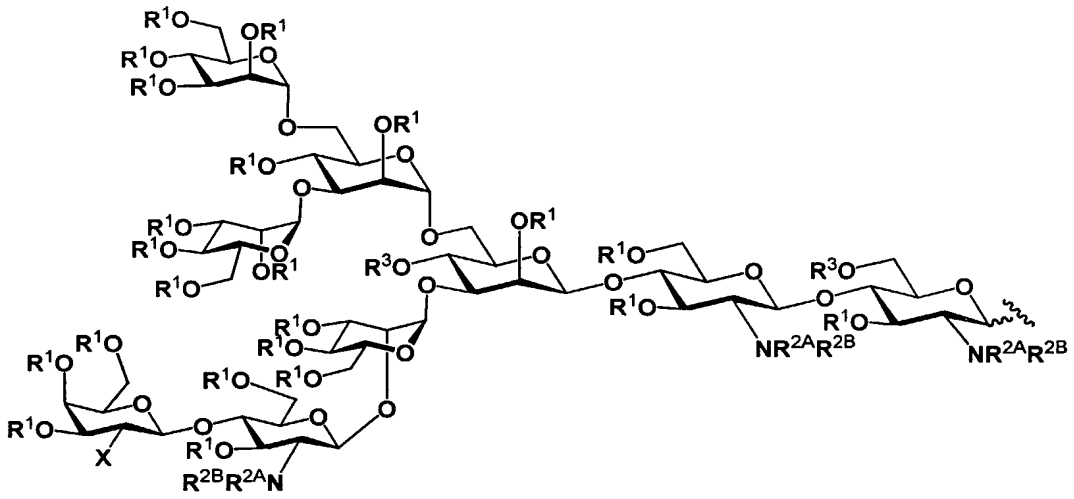


(I^{det})



(II^{det})

or

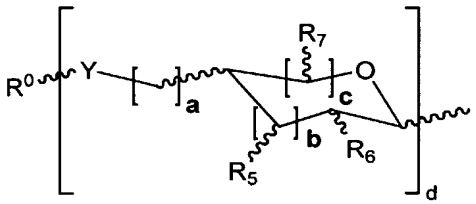


(III^{det})

wherein each occurrence of R^1 is independently hydrogen or an oxygen protecting group;

each occurrence of R^{2A} and R^{2B} is independently hydrogen or a nitrogen protecting group;

each occurrence of R^3 is independently hydrogen, a protecting group or a carbohydrate domain comprising a saccharide moiety having the structure:

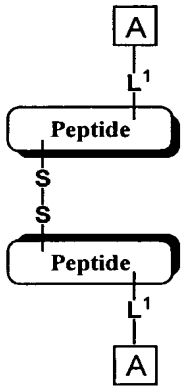


wherein Y is NH or O; wherein a, b and c are each independently 0, 1 or 2; d is an integer from 1-3; with the proviso that the d bracketed structure represents a furanose or pyranose moiety and the sum of b and c is 1 or 2; wherein R^0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein each occurrence of R^5, R^6 and R^7 is independently hydrogen, OH, OR^i, $NR^{ii}R^{iii}$, $NHCOR^i$, F, CH_2OH, CH_2OR^i, or a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of R^i, R^{ii} and R^{iii} is independently hydrogen, a protecting group, a sialic acid moiety, CHO, $COOR^{iv}$, or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group, or R^{ii} and

R^{iii}, taken together with the nitrogen atom to which they are attached, form a substituted or unsubstituted heterocyclic or heteroaryl moiety; and wherein each occurrence of R^{iv} is independently H, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group; and

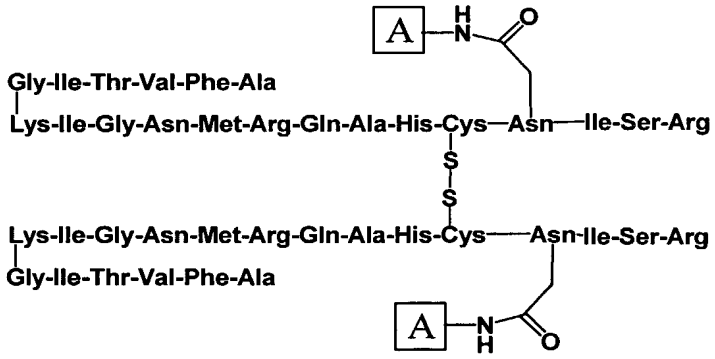
W^1, W^2 and W^3 are independently optionally substituted mannose, galactose or lactosamine moieties.

[0231] In certain embodiments, the invention provides an antibody or antibody fragment which is specific to any one or more of the inventive antigens present on a dimeric glycopeptide having the structure:

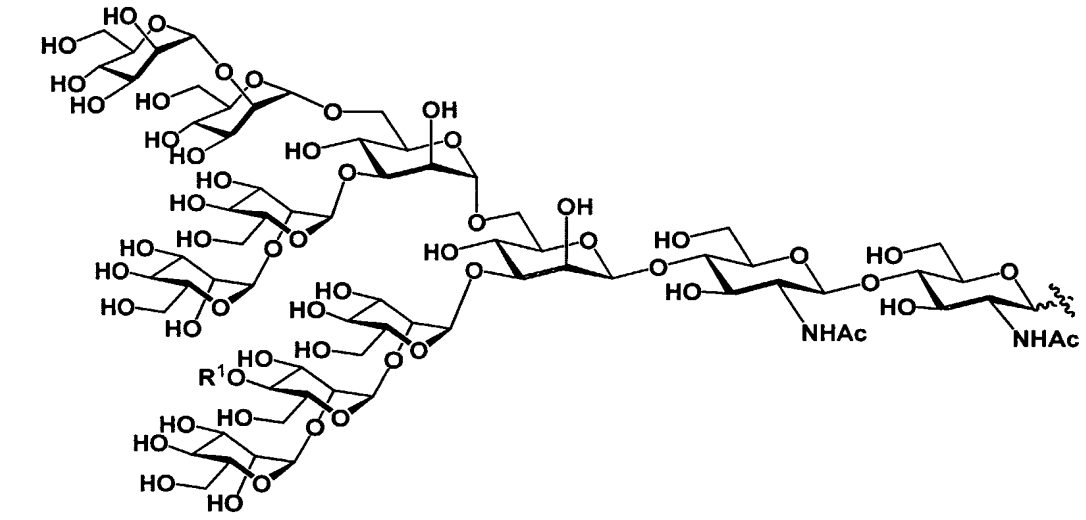


wherein each peptide may be the same or different; and each occurrence of A is independently a carbohydrate domain of formula **(I^{det})**, **(II^{det})** or **(III^{det})**.

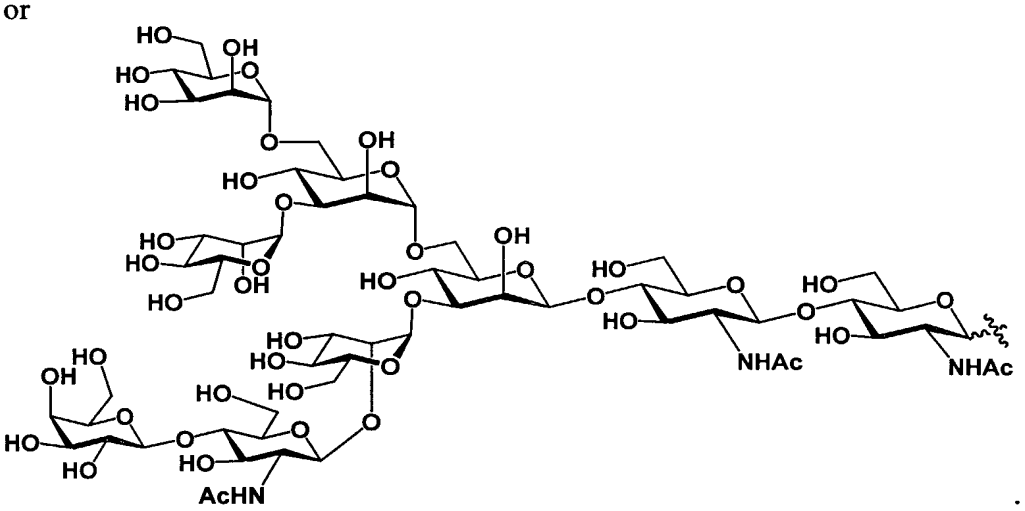
[0232] In certain embodiments, the antigen has the structure:



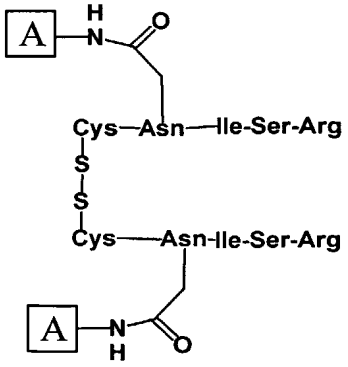
wherein each occurrence of A is independently a carbohydrate domain having one of the structures:



or

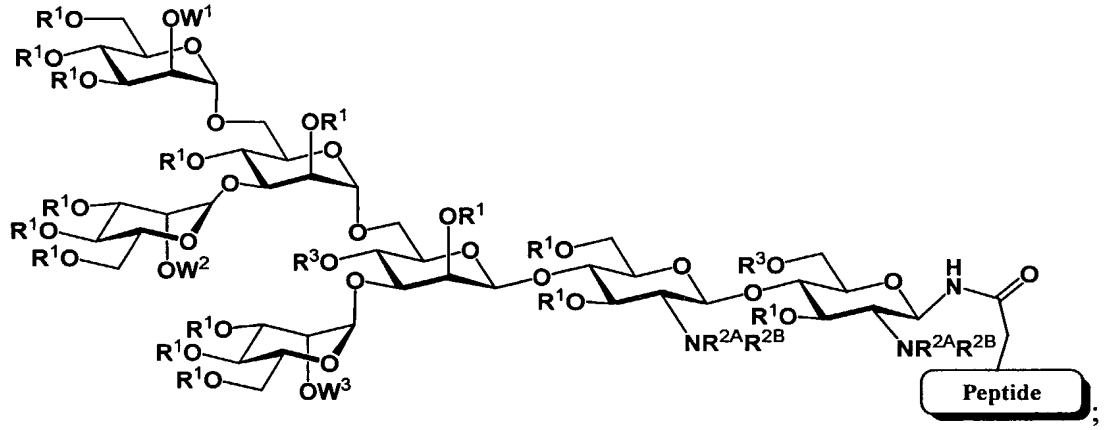


[0233] In certain embodiments, the antigen has the structure:



wherein A is as defined above.

[0234] In yet other embodiments, the antigen comprises a carbohydrate antigen having the structure:

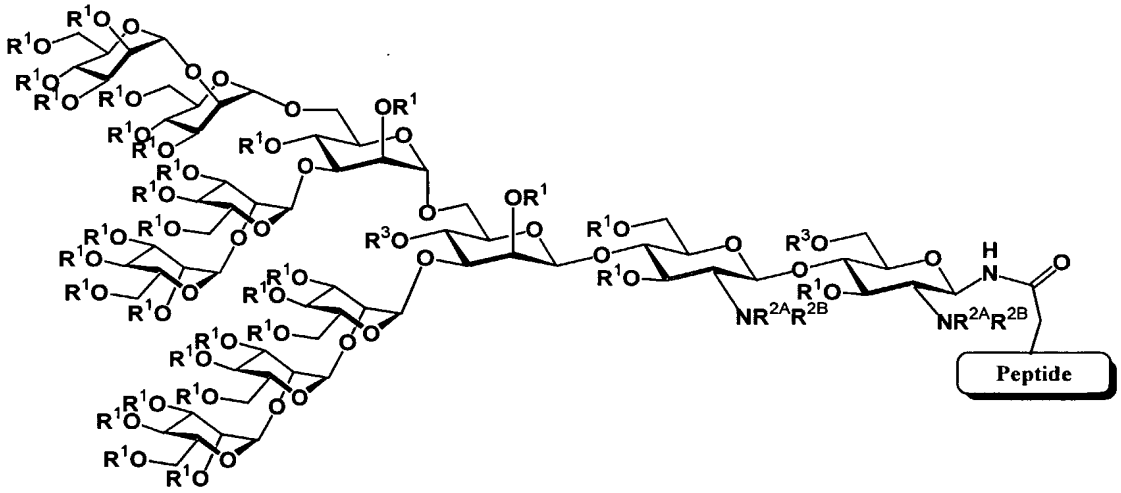


;

(I^A)

wherein the peptide has a structure either identical to or closely related to that of gp120 near an N-glycosylation site.

[0235] In certain embodiments, the invention provides an antibody or antibody fragment which is specific to a compound of formula **(II^A)** having the structure:



;

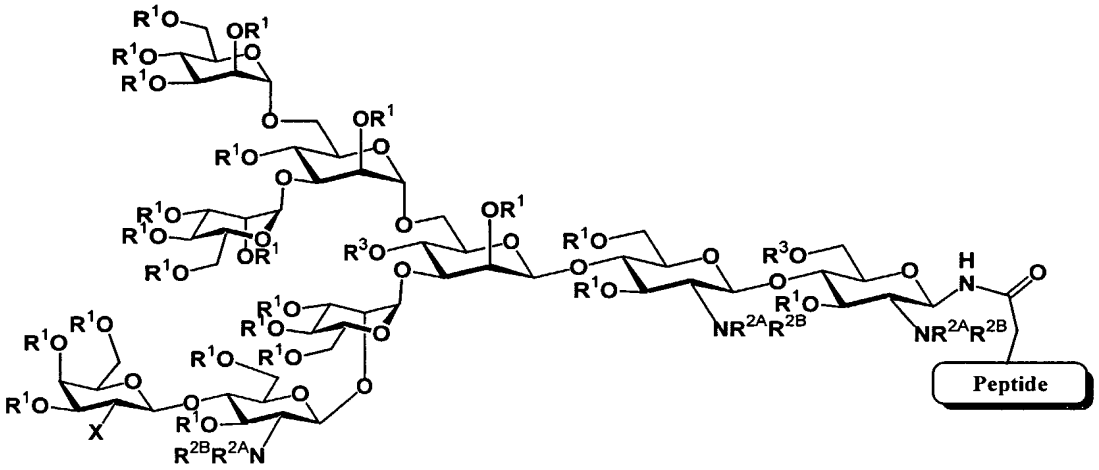
(II^A)

wherein each occurrence of R^1 is independently hydrogen or an oxygen protecting group; each occurrence of R^{2A} and R^{2B} is independently hydrogen or a nitrogen protecting group; and each occurrence of R^3 is independently hydrogen or a protecting group;

wherein the peptide has a structure either identical to or closely related to that of gp120 near an N-glycosylation site;

and wherein said antibody is a purified polyclonal antibody or a monoclonal antibody.

[0236] In certain embodiments, the invention provides an antibody or antibody fragment which is specific to a compound of formula **(III^A)** having the structure:



;

(III^A)

wherein each occurrence of R^1 is independently hydrogen or an oxygen protecting group; each occurrence of R^{2A} and R^{2B} is independently hydrogen or a nitrogen protecting group; and each occurrence of R^3 is independently hydrogen or a protecting group;

wherein the peptide has a structure either identical to or closely related to that of gp120 near an N-glycosylation site;

and wherein said antibody is a purified polyclonal antibody or a monoclonal antibody.

[0237] In certain exemplary embodiments, the antibody is a monoclonal antibody.

[0238] The compounds of the invention may be used to prepare monoclonal or polyclonal antibodies. Conventional methods can be used to prepare the antibodies. As to the details relating to the preparation of monoclonal antibodies reference can be made to Goding, J. W., Monoclonal Antibodies: Principles and Practice, 2nd Ed., Academic Press, London, 1986.

[0239] The compounds, as well as antibodies specific for the inventive gp120 glycans and/or constructs thereof may be labelled using conventional methods with various enzymes, fluorescent materials, luminescent materials and radioactive material. Linking an antibody or an antibody fragment to a label, whether it is a radioactive, enzymatic or colored label or any other type of label commonly used in immunological techniques, is well known and described in the literature. Suitable enzymes, fluorescent materials, luminescent materials, and radioactive material are well known to the skilled artisan.

[0240] It is presently unknown, however, how large a segment of gp120 is required to generate appropriate antibodies; e.g., the glycopeptide may not have enough native structure to develop appropriately specific antibodies. The glycopeptide might not itself be immunogenic, and could therefore require the use of an adjuvant to stimulate an immune response. Examples of suitable adjuvants include, but are not limited to, saponin adjuvants (*e.g.*, GPI-0100), *Salmonella minnesota* cells, bacille Calmette-Guerin and/or QS21.

[0241] A lack of immune response with any length glycopeptide would call for the use of a carrier protein such as keyhole limpet hemocyanin (KLH), [34-36] an adjuvant [37] such as covalently bound Pam_3Cys, [38] or coadministered QS21. [39] Such immunostimulants have been used alone or in concert[40-42] to generate antibodies from small glycopeptide haptens, [43-45] and should prove effective here, as well. Though the first two systems require covalent conjugation, the synthetic design allows late-stage conjugation as demonstrated previously for other glycopeptides.[46]

[0242] **References**

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antibody titers against ovine submaxillary mucin and sialyl-Tn-positive tumor cells." *Cancer Immunol. Immun.* **1999**, *48*, 1-8.

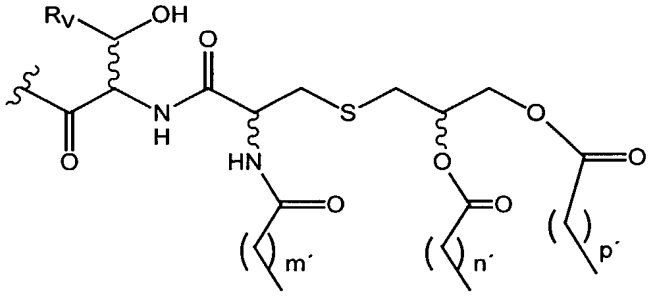
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[0243] *Methods of Treatment*

[0244] As detailed above, a major drawback in using carbohydrate epitopes, is that they are generally not readily available by isolation from natural sources. For example, the immense difficulties associated with their purification from natural sources render them virtually nonavailable as homogeneous starting materials for a clinical program. Thus, the incorporation of these naturally occurring epitopes into carrier proteins/peptides or any favorable molecular context via conjugation for eliciting a therapeutically useful immunological response is inefficient at best, and often virtually impossible. Therefore, to effectively study vaccines as therapeutic agents, sufficient material can only be obtained by chemical synthesis. As discussed above, the present invention provides a variety of synthetic glycoforms of gp120 (glycans glycopeptide conjugates and/or other constructs thereof), and methods for preparing them.

[0245] Accordingly, in another aspect of the invention, a method of treatment is provided comprising administering to a subject in need thereof a therapeutically effective amount of any of the gp120 glycans and/or glyconjugates thereof disclosed herein (*e.g.*, glycopeptides, which may additionally be conjugated to a protein, peptide or lipid carrier, either directly or through a crosslinker), optionally in combination with a pharmaceutically acceptable carrier. In certain embodiments, a method for preventing the infection with HIV is provided comprising administering to a subject in need thereof a therapeutically effective amount of any of the gp120 glycans and/or glyconjugates thereof disclosed herein, optionally in combination with an adjuvant. In certain embodiments, a method for the treatment of HIV is provided comprising administering to a subject in need thereof a therapeutically effective amount of any of the gp120 glycans and/or

glyconjugates thereof disclosed herein, optionally in combination with an adjuvant. In certain embodiments, a method for inducing antibodies in a human subject, wherein the antibodies are specific to a carbohydrate antigen expressed on the surface of gp120, which comprises administering to the subject an amount of any of the glycans and/or glycoconjugates disclosed above effective to induce antibodies. In certain embodiments, the method utilized any one or more of the gp120 glycans and/or glycoconjugates thereof disclosed herein, where the glycan(s) and/or glycoconjugate(s) is/are linked to an immunogenic carrier either directly or through a crosslinker, which carrier is a protein, peptide or lipid. In certain embodiments, the carrier is Bovine Serum Albumin, polylysine or KLH. In certain other embodiments, the carrier is a lipid having the structure:



wherein m′, n′ and p′ are each independently integers between about 8 and 20; and R_V is hydrogen, substituted or unsubstituted linear or branched chain lower alkyl or substituted or unsubstituted phenyl. In certain exemplary embodiments, m′, n′ and p′ are each 14 and the lipid is tripalmitoyl-S-glycerylcysteinylserine (*e.g.*, PamCys).

[0246] In certain other embodiments, the method comprises administering to a subject in need thereof a therapeutically effective amount of any of the compounds and/or glycoconjugates disclosed herein, in combination with an immunogenic carrier, optionally in combination with a pharmaceutically acceptable carrier. Specifically, in certain exemplary embodiments, the method comprises administering a gp120 glycan and/or glycoconjugate thereof additionally conjugated to an immunogenic carrier. In certain embodiments, the method comprises administering to the subject a therapeutically effective amount of any one or more of the glyconjugates disclosed herein (*e.g.*, glycopeptides, which may additionally be conjugated to a protein, peptide or lipid carrier, either directly or through a

crosslinker), in combination with an immunogenic carrier, optionally in combination with a pharmaceutically acceptable carrier. In certain embodiments, the method comprises administering one or more gp120 glycans and/or glycoconjugates and an immunogenic carrier that have not been conjugated. Rather, they are administered concurrently, or successively, as separate entities. In certain other exemplary embodiments, the method comprises administering one or more gp120 glycans and/or glycoconjugates of the invention conjugated (*i.e.*, covalently linked) to an immunogenic carrier. In certain embodiments, the method comprises administering any one or more inventive gp120 glycans and/or glycoconjugates thereof disclosed herein that have not been conjugated to an immunogenic carrier. Rather, the gp120 glycan(s) and/or glycoconjugate(s) thereof and the immunogenic carrier are administered concurrently, or successively, as separate entities. In certain embodiments, the immunogenic carrier is a protein, peptide or lipid. In certain exemplary embodiments, the carrier is Bovine Serum Albumin, polylysine or KLH. In certain other embodiments, the carrier is PamCys. For the purpose of the invention, a compound/glycoconjugate and a carrier are said to be administrered concurrently when they are administered (i) as a single composition containing the compound/glycoconjugate and the carrier, (ii) as two separate compositions or (iii) are delivered by separate routes within a short enough period of time that the effective result is equivalent to that obatined when both compound/ glycoconjugate and carrier are administered as a single composition.

[0247] In still other embodiments, the present invention provides the related method of inducing antibodies which further comprises co-administering an immunological adjuvant, or a combination of immunological adjuvants.

[0248] In certain exemplary embodiments, the inventive gp120 glycans and glycoconjugates thereof comprise carbohydrate domains, or truncated or elongated versions thereof, that are found on the surface of gp120. In certain exemplary embodiments, the inventive glycoconjugates comprise peptidic domains, or truncated or elongated versions thereof, that are found near an N-glycosylation site of naturally occurring gp120.

[0249] Accordingly, embodiments of this invention encompass methods of eliciting immune responses in animals comprising administering effective amounts

of inventive gp120 glycans and/or glycoconjugate(s) thereof and/or compositions of the invention wherein the immune response is directed against on eor more carbohydrates expressed on the surface of gp120.

[0250] A further embodiment of this invention encompasses a use of effective amounts of inventive gp120 glycans and/or glycoconjugate(s) thereof and/or a composition of the present invention to elicit an immune response in an animal preferably to treat and/or prevent HIV. The present invention further includes a use of effective amounts of inventive gp120 glycans and/or glycoconjugate(s) thereof and/or a composition of the present invention to prepare a medicament to elicit an immune response in animal, preferably to treat and/or prevent HIV.

[0251] It will be appreciated that the magnitude of the therapeutic dose of the compounds of the invention will vary with the nature and severity of the condition to be treated and with the particular compound and its route of administration. In general, the daily dose range for antiHIV activity lies in the range of 0.0001 to 1.0 mg/kg of body weight in a mammal, although the present invention is not intended to be limited by this range.

[0252] Any suitable route of administration may be employed for providing a mammal, especially a human, with an effective dosage of a compound disclosed herein. For example, oral, rectal, topical, parenteral, ocular, pulmonary, nasal, etc. routes may be employed. Dosage forms include tablets, troches, dispersions, suspensions, solutions, capsules, creams, ointments, aerosols, etc. In preferred embodiments, the effective dosage is employed using a syringe injection.

[0253] It will be appreciated by one of ordinary skill in the art, however, that the most suitable route for administration will depend largely on the nature and severity of the condition being treated and on the nature of the active ingredient. As discussed above, the inventive therapeutics may be conveniently presented in unit dosage form and prepared by any of the methods well known in the art of pharmacy.

[0254] Additionally, once a synthetic vaccine has been derivatized and characterized, mouse immunological studies can be performed to assess the potency and/or specificity of the novel HIV vaccines.

KITS OF THE INVENTION

[0255] In other embodiments, the present invention relates to a kit for conveniently and effectively carrying out the methods in accordance with the present invention. In general, the pharmaceutical pack or kit comprises one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Such kits are especially suited for the delivery of solid oral forms such as tablets or capsules. Such a kit preferably includes a number of unit dosages, and may also include a card having the dosages oriented in the order of their intended use. If desired, a memory aid can be provided, for example in the form of numbers, letters, or other markings or with a calendar insert, designating the days in the treatment schedule in which the dosages can be administered. Alternatively, placebo dosages, or calcium dietary supplements, either in a form similar to or distinct from the dosages of the pharmaceutical compositions, can be included to provide a kit in which a dosage is taken every day. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceutical products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

EQUIVALENTS

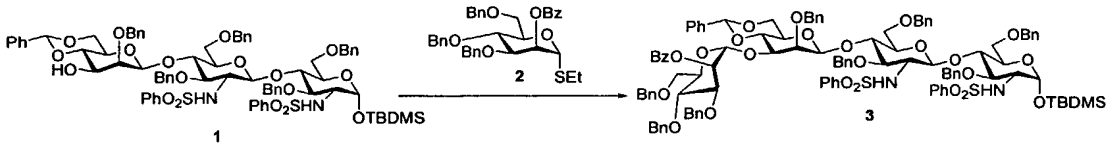
[0256] The representative examples which follow are intended to help illustrate the invention, and are not intended to, nor should they be construed to, limit the scope of the invention. Indeed, various modifications of the invention and many further embodiments thereof, in addition to those shown and described herein, will become apparent to those skilled in the art from the full contents of this document, including the examples which follow and the references to the scientific and patent literature cited herein. In but one illustrative example, protecting groups play an important role in the synthesis of the carbohydrate domains and synthetic conjugates, as described herein; however it will be appreciated by one of ordinary skill in the art that the present invention encompasses the use of various alternate protecting groups known in the art. Those protecting groups used in the disclosure including the Examples below are merely illustrative.

[0257] It should further be appreciated that, uless otherwise indicated, the contents of those cited references are incorporated herein by reference to help illustrate the state of the art. The following examples contain important additional information, exemplification and guidance which can be adapted to the practice of this invention in its various embodiments and the equivalents thereof.

EXEMPLIFICATION

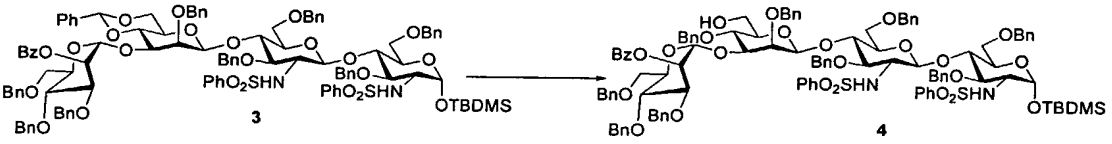
[0258] **Gp120 glycans and glycopeptides**

[0259] General Methods: Reagents obtained from commercial suppliers were used without further purification unless otherwise noted. THF, toluene, and methylene chloride was obtained from a dry solvent system (passed through a prepacked column of alumina) and used without further drying. All air and water sensitive reactions were performed in flame-dried glassware under a positive pressure of prepurified argon gas. NMR (^1H and ^{13}C) spectra were recorded on Bruker AMX-400 MHz or Bruker Advance DRX-500 MHz as noted individually, referenced to $CDCl_3$ (7.27 ppm for ^1H and 77.0 ppm for ^{13}C) or CD_3COCD_3 (2.09 ppm for ^1H and 30.6 and 205.9 ppm for ^{13}C). Optical rotations were obtained on a JASCO model DIP-370 digital polarimeter. Analytical thin-layer chromatography was performed on E. Merck silica gel 60 F254 plates. Compounds which were not UV active were visualized by dipping the plates in para-anisaldehyde solution and heating. Preparative thin layer chromatography was performed using the indicated solvent on Whatman® (LK6F Silica gel 60 Å 250 μM or Pk6F Silica Gel 60 Å 1000 μM) TLC plate.

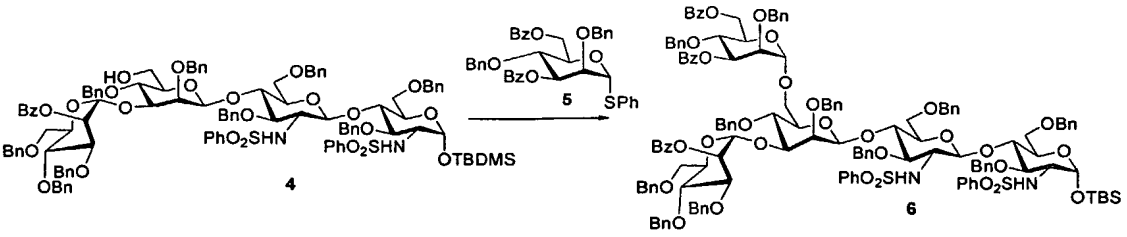


[0260] **Tetrasaccharide 3**: A mixture of trisacchride **1**[1] (106 mg, 0.074 mmol), thiomannoside **2** (133 mg, 0.222 mmol) and molecular sieves in CH_3CN (2 mL) was stirred for 2 h at r.t. and tris(4-bromophenyl)aminium hexachloroantimonate (199 mg, 0.244 mmol) was added at 15 °C. The solution was stirred for 4 h at r.t. and then quenched by triethylamine. The mixture was filtered

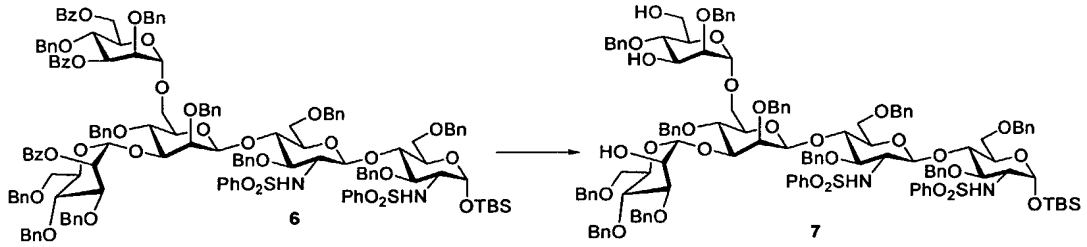
through celite, concentrated, dissolved in EtOAc, filtered through silica gel and concentrated. The residue was purified by preparative TLC (PTLC) using pentane/ether (1/2) as the eluent to afford **3** as a white solid (113 mg, 78%). $[\alpha]_D^{25}$ -205.0 (*c* 0.14, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ 0.00 (s, 3 H), 0.06 (s, 3 H), 0.87 (s, 9 H), 5.07 (s, 1 H), 5.30 (s, 1 H), 5.36 (s, 1 H), 5.74 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.8, -4.6, 13.9, 17.8, 20.8, 25.6, 57.8, 58.6, 60.1, 66.8, 67.5, 67.8, 68.2, 68.4, 68.9, 69.5, 71.0, 72.3, 73.1, 73.3, 73.5, 73.9, 74.3, 74.9, 75.1, 75.3, 75.8, 77.3, 77.6, 77.9, 78.2, 79.9, 92.6, 98.4, 100.7, 100.9, 125.6, 126.7, (126.8-129.5), 129.6, 136.9, 137.4, 137.6, 138.0, 138.1, 138.2, 138.3, 140.4, 141.3, 165.1. LRMS (ESI) calcd for $C_{112}H_{122}N_2O_{24}S_2SiNa^+$ $[M+Na]^+$ 1994.76, found 1994.8.



[0261] **Tetrasaccharide 4:** To a solution of **3** (200 mg, 0.101 mmol) in borane tetrahydrofuran etherate (1.1 mL, 1.0 M in THF, 1.01 mmol) was added dibutylboron triflate (0.334 mL, 1.0 M in CH_2Cl_2, 0.333 mmol) at 0 °C. The reaction mixture was stirred for 7 h at 0 °C and quenched with triethylamine and methanol and concentrated. The residue was purified by PTLC using pentane/ether (1/2) as the eluent to afford **4** as a white solid (172 mg, 90%). $[\alpha]_D^{25}$ -187.0 (*c* 0.13, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ -0.08 (s, 3 H), -0.04 (s, 3 H), 0.80 (s, 9 H), 4.96 (d, J = 2.6 Hz, 1 H), 5.15 (s, 1 H), 5.55 (s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.7, -4.6, 17.9, 25.7, 57.9, 58.3, 67.6, 68.9, 69.8, 71.3, 72.4, 73.2, 73.4, 73.9, 74.3, 74.5, 75.0, 76.0, 77.3, 78.1, 79.6, 79.9, 92.7, 99.3, 100.6, 101.0, 126.8-128.7, 129.8, 137.6, 137.7, 138.2, 138.3, 138.4, 140.5, 141.0, 165.2. LRMS (ESI) calcd for $C_{112}H_{124}N_2O_{24}S_2SiNa^+$ $[M+Na]^+$ 1995.8, found 1995.8.

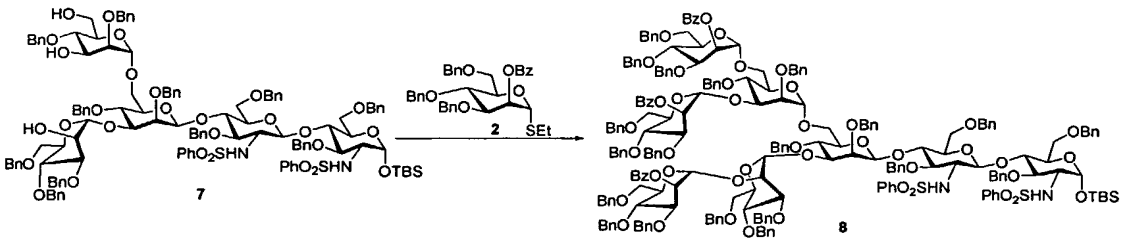


[0262] **Pentasaccharide 6:** 6 was prepared using same procedure as the synthesis of **3**. White solid (80 mg, 74%). $[\alpha]_D^{25}$ 51.0 (*c* 0.13, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ -0.07 (s, 3 H), -0.02 (s, 3 H), 0.80 (s, 9 H), 4.95 (s, 1 H), 4.99 (s, 1 H), 5.25 (s, 1 H), 5.54 (dd, *J* = 9.5, 2.5 Hz, 1 H), 5.58 (s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.8, -4.6, 14.0, 17.9, 20.9, 22.5, 25.7, 31.4, 57.8, 58.6, 60.2, 67.7, 68.9, 69.6, 70.1, 71.5, 72.5, 72.9, 73.2, 73.9, 74.4, 74.8, 75.2, 75.9, 76.4, 77.3, 79.1, 92.7, 97.9, 99.4, 101.1, 126.9-129.5, 129.6, 137.3, 137.7, 138.2, 138.4, 141.1, 165.2, 165.5, 166.1. LRMS (ESI) calcd for $C_{146}H_{154}N_2O_{31}S_2SiNa^+$ $[M+Na]^+$ 2546.0, found 2545.9.

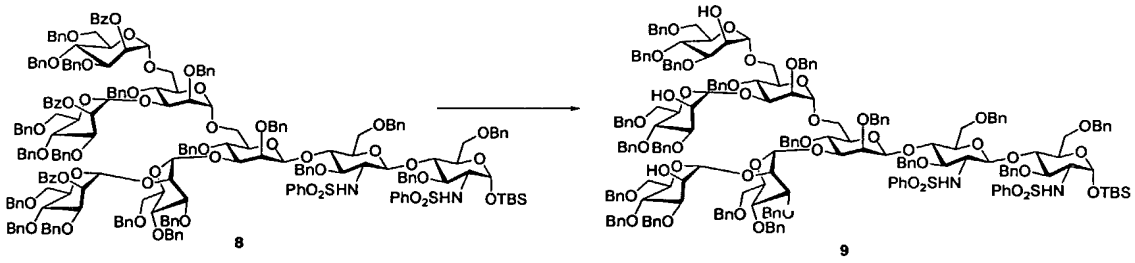


[0263] **Pentasaccharide triol 7:** To a solution of **6** (80 mg, 0.032 mmol) in MeOH (2 mL) was added sodium methoxide in MeOH (25%, 0.1 mL) and stirred for 12 h and quenched with NH_4Cl saturated aqueous solution and concentrated. The residue was dissolved in EtOAc and washed with water and brine. The organic layer was dried with anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by PTLC using pentane/ether (1/3) as the eluent to afford **7** as a white solid (64 mg, 91%). $[\alpha]_D^{25}$ 121.8 (*c* 0.16, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ 0.00 (s, 3 H), 0.05 (s, 3 H), 0.93 (s, 9 H), 4.92 (s, 1 H), 5.06 (d, *J* = 1.8 Hz, 1 H), 5.14 (s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.8, -4.6, 14.0, 17.9, 20.9, 25.7,

57.8, 58.4, 60.2, 65.9, 68.4, 69.6, 71.6, 71.9, 72.4, 72.6, 73.1, 73.2, 73.3, 73.9, 74.1, 74.4, 74.7, 74.8, 74.9, 75.3, 75.8, 76.1, 76.4, 77.3, 78.3, 79.1, 79.6, 80.6, 92.7, 97.2, 100.5, 101.2, 101.3, 126.8-128.6, 137.6, 137.8, 137.9, 138.2, 138.3, 138.6, 140.5, 141.1. LRMS (ESI) calcd for $C_{125}H_{142}N_2O_{28}S_2SiNa^+$ [M+Na^+] 2233.9, found 2233.9.

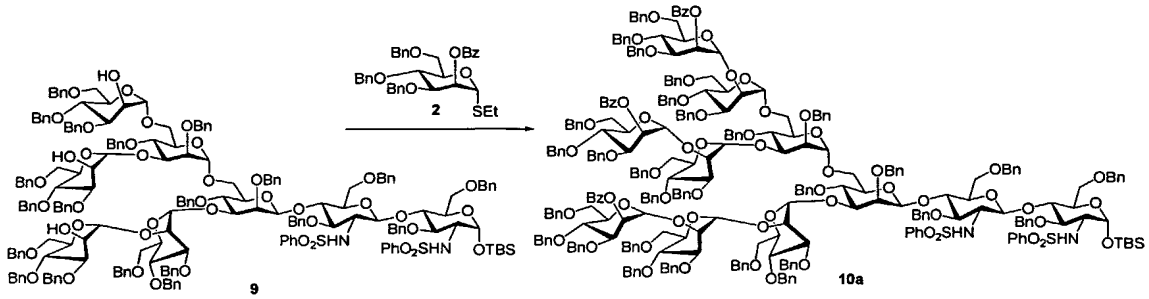


[0264] **Octasaccharide 8**: **8** was prepared following the same protocol as used for **3** using thiol mannoside donor **2** as excess (10 eq.) White solid: (61 mg, 55%). $[\alpha]_D^{25}$ 32.8 (*c* 0.15, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ 0.00 (s, 3 H), 0.05 (s, 3 H), 0.88 (s, 9 H), 4.79 (s, 1 H), 4.88 (s, 1 H), 5.01 (s, 1 H), 5.06 (s, 1 H), 5.23 (s, 1 H), 5.58 (s, 1 H), 5.62 (s, 1 H), 5.66 (s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.1, -3.9, 14.7, 18.5, 21.5, 26.3, 30.2, 58.4, 59.1, 60.9, 66.5, 69.0, 69.3, 69.4, 70.3, 71.3, 72.0, 72.2, 72.6, 72.8, 73.6, 73.7, 73.8, 74.0, 74.6, 75.1, 75.3, 75.5, 75.7, 78.4, 78.9, 80.4, 82.2, 93.3, 98.2, 98.9, 99.7, 101.2, 101.8, 102.8, 126.9-130.5, 138.5, 138.6, 138.8, 139.0, 139.1, 139.6, 141.2, 165.8, 165.9. LRMS (ESI) calcd for $C_{227}H_{238}N_2O_{46}S_2SiNa_2$ [M+2Na]$^{2+}$ 1932.8, found 1933.0.

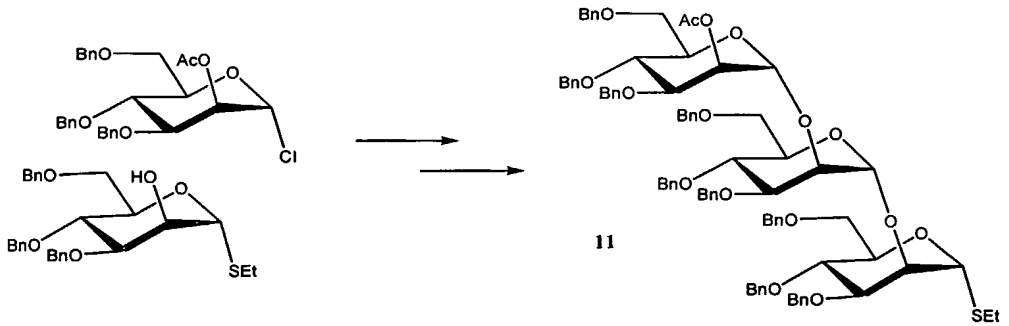


[0265] **Octasaccharide triol 9:** The synthesis of 9 follows the synthetic procedure of **7**. White solid (46 mg, 87%). $[\alpha]_D^{25}$ 280.0 (*c* 0.12, $CHCl_3$). ^1H NMR

(400 MHz, $CDCl_3$) selected signals: δ -0.08 (s, 3 H), -0.03 (s, 3 H), 0.80 (s, 9 H), 4.92 (s, 1 H), 4.94 (s, 1 H), 4.97 (s, 1 H), 5.03 (s, 1 H), 5.07 (s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.7, -4.4, 0.0, 14.1, 18.0, 22.7, 25.8, 29.3, 29.7, 31.9, 58.0, 58.6, 65.5, 66.3, 67.7, 68.4, 68.5, 68.8, 71.1, 71.2, 71.7, 71.8, 72.0, 72.3, 72.9, 73.2, 73.3, 73.5, 74.1, 74.2, 74.3, 74.5, 74.8, 74.9, 75.0, 79.4, 81.8, 92.8, 97.4, 100.0, 100.7, 100.9, 101.3, 102.9, 127.1-128.8, 138.0-138.5, 140.7, 141.3. LRMS (ESI) calcd for $C_{206}H_{226}N_2O_{43}S_2SiNa_2$ $[M+2Na]^{2+}$ 1776.7, found 1776.7.

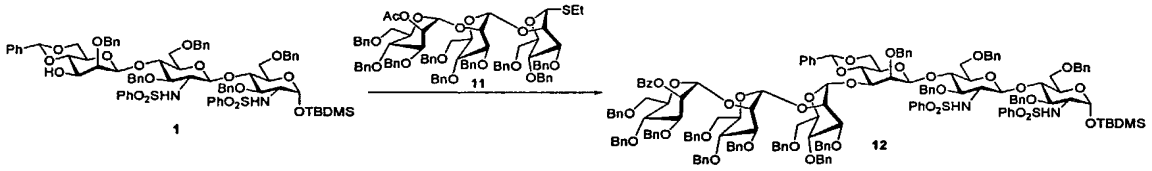


[0266] **Undecasaccharide 10a**: The synthesis of **10a** follows same synthetic procedure as **8**. **10a**, white solid (81 mg, 51%). $[\alpha]_D^{25}$ 73.8 (*c* 0.09, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ -0.05 (s, 3 H), -0.00 (s, 3 H), 0.82 (s, 9 H), 5.00-5.20 (m, 7 H), 5.65-5.68 (m, 3 H). LRMS (ESI) calcd for $C_{308}H_{322}N_2O_{61}S_2SiNa_2$ $[M+2Na]^{2+}$ 2581.1, found 2581.3.

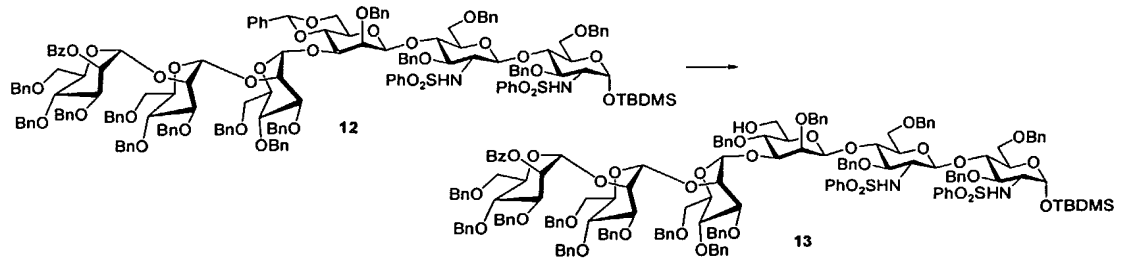


[0267] **Trisaccharide donor 11:** Trisaccharide donor **11** was prepared from the known chloride and thiomannoside monosaccharides according to standard coupling procedures. ^{1} H NMR ($CDCl_3$, 400 MHz) δ: 1.21 (t, J = 7.5 Hz, 3H), 2.16 (s, 3H), 2.47-2.57 (m, 2H), 3.57 (d, J = 10.7 Hz, 1H), 3.67-3.75 (m, 4H), 3.78-3.85

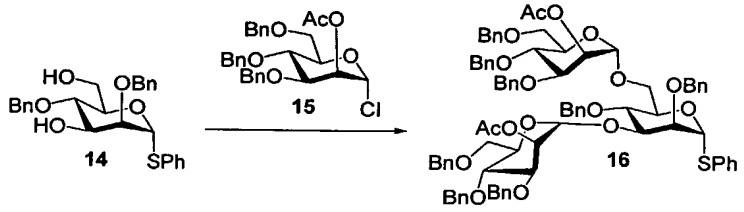
(m, 4H), 3.89-3.97 (m, 3H), 3.99-4.04 (m, 2H), 4.08-4.12 (m, 3H), 4.36 (d, J = 12.2 Hz, 1H), 4.43-4.73 (m, 14H), 4.82-4.88 (m, 3H), 5.08 (d, J = 2.0 Hz, 1H), 5.20 (d, J = 2.0 Hz, 1H), 5.46 (d, J = 1.3 Hz, 1H), 5.56 (dd, J = 3.0, 1.9 Hz, 1H), 7.14-7.38 (m, 45H).



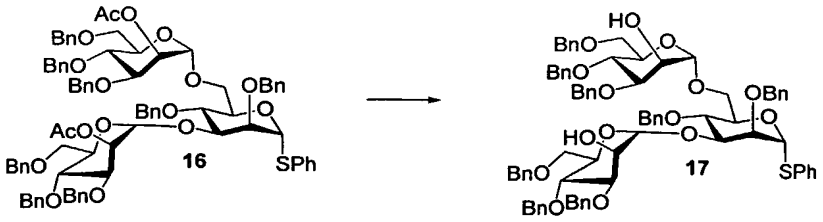
[0268] **Hexasaccharide 12**: To a mixture of **1** (35 mg, 0.024 mmol), **11** (51 mg, 0.037 mmol) and molecular sieves in CH_2Cl_2 (2 mL) was added di-*tert*-butylpyridine (DTBP) (0.019 mL, 0.085 mmol) at -40 °C and stirred for 1 h at -40 °C. MeOTf (0.011 mL, 0.096 mmol) was added and the reaction mixture was warmed up to r.t. and stirred for 12 h before quenched with triethylamine, filtered through celite, washed with $NaHCO_3$ saturated aqueous solution, brine, dried over anhydrous $MgSO_4$ and filtered. The organic layer was concentrated and residue purified by PTLC using pentane/ether (1/1.3) as the eluent to afford **12** as a white solid (47 mg, 47%). $[\alpha]_D^{25}$ 41.4 (*c* 0.65, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ 0.91 (s, 9 H), 4.97 (s, 1 H), 5.11 (s, 1 H), 5.21 (s, 1 H), 5.25 (s, 1 H), 5.28 (s, 1 H), 5.52 (s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.7, -4.4, 0.0, 14.2, 18.0, 21.0, 21.1, 25.8, 58.0, 58.8, 60.4, 67.0, 68.6, 68.7, 69.8, 71.6, 71.9, 72.1, 72.9, 73.3, 73.7, 73.8, 74.4, 74.6, 75.1, 75.4, 75.5, 76.0, 77.4, 78.3, 78.8, 80.2, 92.9, 99.5, 99.9, 100.2, 100.8, 101.0, 101.1, 125.9, 127.1-128.5, 137.9, 138.1, 138.4, 138.6, 138.8, 140.7, 170.0, 171.1. LRMS (ESI) calcd for $C_{161}H_{176}N_2O_{34}S_2SiNa_2$ $[M+2Na]^{2+}$ 1409.6, found 1409.4.



[0269] **Hexasaccharide 13:13** was prepared using the same procedure as the one for **4**. **13**, white solid (542 mg, 86%). $[\alpha]_D^{25}$ 91.5 (*c* 0.54, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ 0.02 (s, 3 H), 0.04 (s, 3 H), 0.87 (s, 9 H), 5.01 (s, 1 H), 5.06 (s, 1 H), 5.09 (s, 1 H), 5.15 (s, 1 H), 5.49 (s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.7, -4.4, 0.0, 14.2, 18.0, 21.0, 21.1, 21.4, 25.8, 58.0, 58.3, 60.4, 61.3, 67.6, 68.6, 69.9, 71.8, 72.0, 72.1, 73.1, 73.3, 73.5, 74.1, 74.5, 74.6, 74.8, 75.1, 76.1, 76.2, 78.2, 78.7, 79.8, 81.1, 92.8, 99.4, 100.5, 100.7, 101.0, 101.3, 125.3, 127.0-128.5, 137.9, 138.0, 138.4, 138.5, 138.6, 138.7, 140.7, 170.1. LRMS (ESI) calcd for $C_{161}H_{178}N_2O_{34}S_2SiNa_2$ $[M+2Na]^{2+}$ 1410.6, found 1410.4.

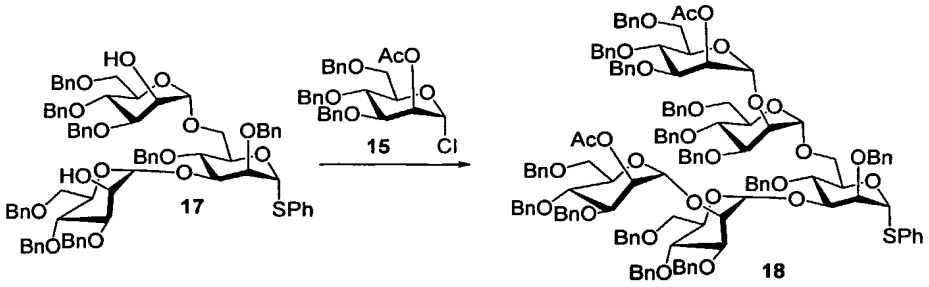


[0270] **Tirsaccharide 16**: To a 25 mL flask containing donor **15** (169 mg, 0.332 mmol) and acceptors **14** (37 mg, 0.083 mmol) (dried azeotropically with toluene) in 1.5 mL dichloromethane was added activated MS 4Å and the mixture was stirred for 1 h at room temperature. In a separate flask, AgOTf (0.087 gm, 0.332 mmol) and DTBP (0.078 mL, 0.347 mmol) in 1.5 mL dichloromethane were stirred with MS 4Å. After one hour the flask containing the AgOTf / DTBP was cooled to -10 °C and the solution containing mixture of donor and acceptor was added over 5 minutes. The solution was stirred in dark with warming up to room temperature over 18 hr. The reaction mixture was diluted with ethyl acetate and was added aqueous saturated $NaHCO_3$ solution. After stirring for 10 minutes, the reaction mixture was filtered through bed of Celite and the filtrate was washed with water, brine, dried over $MgSO_4$ and evaporated *in vacuo*. The crude product was purified by silica gel column chromatography (10% ethyl acetate/toluene) to afford diacetate **16**. This diacetate was used for next step without further purification.



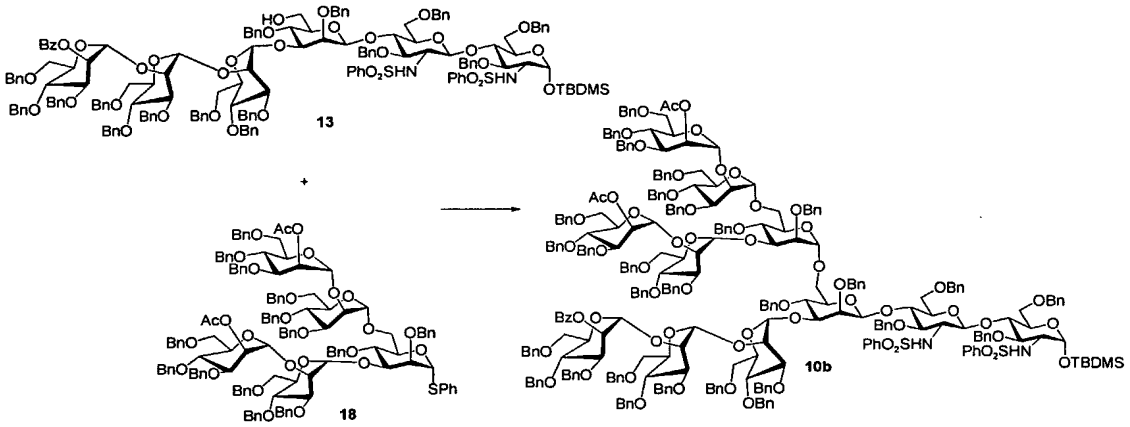
[0271] **Trisaccharide diol 17:**

[0272] **16** was dried azeotropically with toluene and dissolved in 2 mL of anhydrous methanol under argon. Sodium methoxide (25% by weight in methanol, 100 μL) was added and the reaction mixture was stirred for 12 h. Solid ammonium chloride was added and the mixture was stirred for 20 min. The reaction mixture was carefully evaporated to solid residues, and the residues were dissolved in ethyl acetate and washed with brine. Evaporation of ethyl acetate layers provided crude products, which was purified by silica gel column chromatography (10% ethyl acetate/dichloromethane) to yield diol **17** in 50% over two steps. $[\alpha]_D^{25}$ +53.1 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 4.94 (bs, 1H), 5.17 (bs, 1H), 5.44 (bs, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 138.68, 138.66, 138.4, 138.1, 138.06, 138.03, 134.9, 131.0, 129.3, 128.72, 128.70, 128.67, 128.61, 128.49, 128.47, 128.16, 128.13, 128.10, 128.01, 127.96, 127.88, 127.86, 127.83, 127.82, 127.79, 127.76, 127.71, 127.35, 99.9, 85.3, 80.5, 80.2, 79.6, 75.3, 75.15, 75.07, 74.6, 74.4, 73.8, 73.5, 72.7, 72.3, 72.2, 71.8, 71.7, 71.3, 69.5, 68.94, 68.90, 68.2, 66.4. LRMS (ESI) calcd for $C_{80}H_{84}O_{15}SNa^+$ $[M+Na]^+$ 1339.6, found 1339.5.

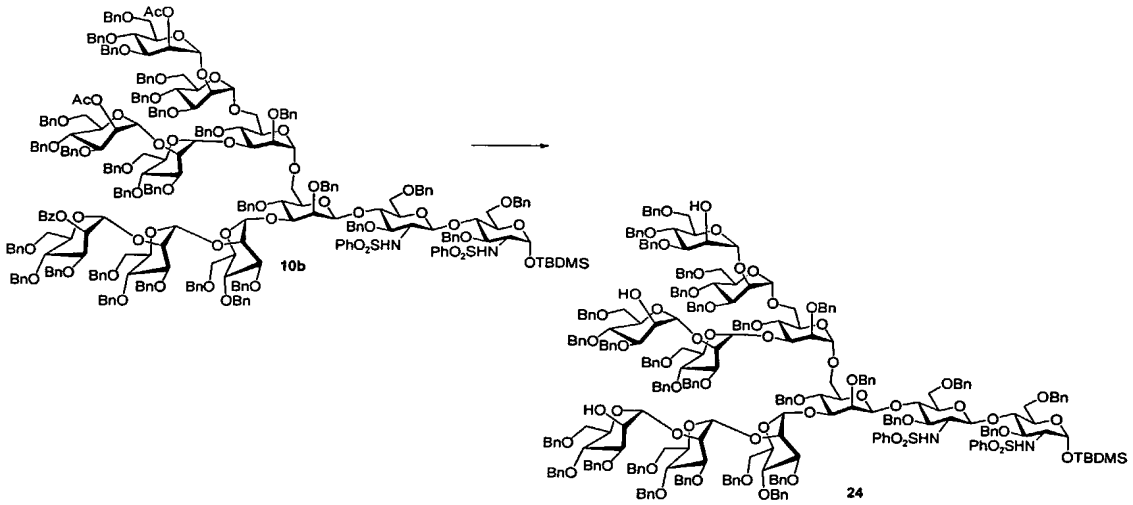


[0273] **Pentasaccharide 18**: To a mixture of **17** (208 mg, 0.158 mmol), **15** (332 mg, 0.631 mmol), molecular sieves, DTBP (0.088 mL, 0.347 mmol) in CH_2Cl_2 (13 mL) was added AgOTf (166 mg, 0.646 mmol) at 0 °C. The mixture was stirred for 18 h at r.t. and quenched with triethylamine, filtered, diluted with EtOAc, washed with $NaHCO_3$ saturated aqueous solution, brine, dried over anhydrous

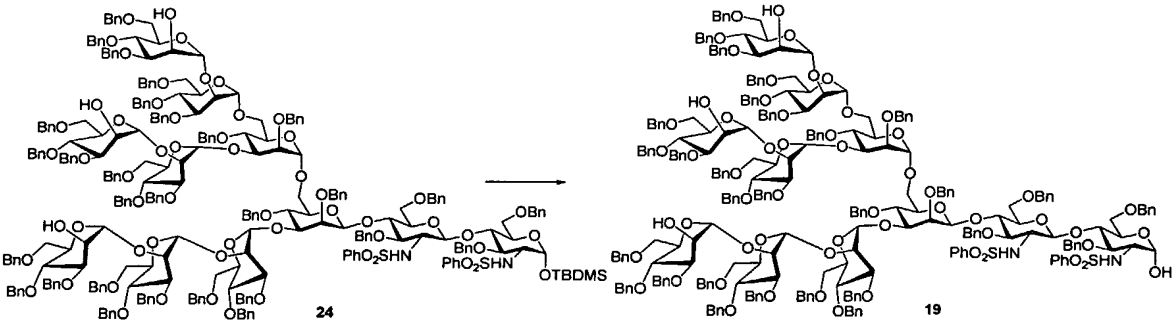
$MgSO_4$ and filtered. The organic layer was concentrated and residue purified by PTLC using pentane/ether (2/1) as the eluent to afford **18** as a white solid (310 mg, 87%). $[\alpha]_D^{25}$ 443.4 (*c* 0.49, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ 2.10 (s, 3 H), 2.11 (s, 3 H), 4.88 (s, 1 H), 5.02 (s, 1 H), 5.04 (s, 1 H), 5.21 (s, 1 H), 5.51 (s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 20.9, 21.0, 21.1, 29.6, 44.6, 60.3, 66.6, 68.7, 68.8, 71.5, 71.7, 71.8, 72.0, 72.1, 73.1, 73.3, 73.4, 74.1, 74.2, 74.4, 74.6, 74.7, 75.0, 75.2, 78.0, 78.1, 79.2, 80.3, 84.8, 89.8, 95.4, 99.0, 99.4, 99.5, 101.2, 116.9, 125.1, 127.1-128.4, 129.1, 130.8, 138.0-138.6, 146.8, 168.3, 170.0. LRMS (ESI) calcd for $C_{138}H_{144}O_{27}SNa^+$ $[M+Na]^+$ 2288.0, found 2287.9.



[0274] **Undecasaccharide 10b**: The preparation of **10b** from **18** and **13** follows the same procedure as the one used for **3**. **10b**, white solid (529 mg, 63% yield, 85% based on recovered starting material). $[\alpha]_D^{25}$ 214.3 (*c* 0.23, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ 0.07 (s, 3 H), 0.15 (s, 3 H), 0.90 (s, 9 H), 2.01 (s, 3 H), 2.10 (bs, 6 H), 5.05 (bs, 1 H), 5.07 (bs, 1 H), 5.10 (bs, 1 H), 5.12 (bs, 1 H), 5.13 (bs, 1 H), 5.15 (bs, 1 H), 5.23 (bs, 1 H), 5.51 (bs, 1 H), 5.54 (bs, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.7, -4.4, 0.0, 1.0, 14.2, 18.0, 21.0, 21.1, 21.2, 25.8, 29.7, 58.0, 58.6, 60.4, 68.6, 68.7, 68.8, 71.8, 72.2, 72.3, 73.0, 73.1, 73.2, 73.3, 74.2, 74.5, 74.8, 75.0, 75.1, 78.2, 78.3, 78.4, 79.4, 92.8, 99.3, 99.5, 100.7, 101.6, 102.3, 127.3-128.4, 138.1-138.7, 140.7, 141.3, 170.0, 170.1, 170.15. LRMS (ESI) calcd for $C_{293}H_{316}N_2O_{61}S_2SiNa_2$ $[M+2Na]^{2+}$ 2488.0, found 2488.0.

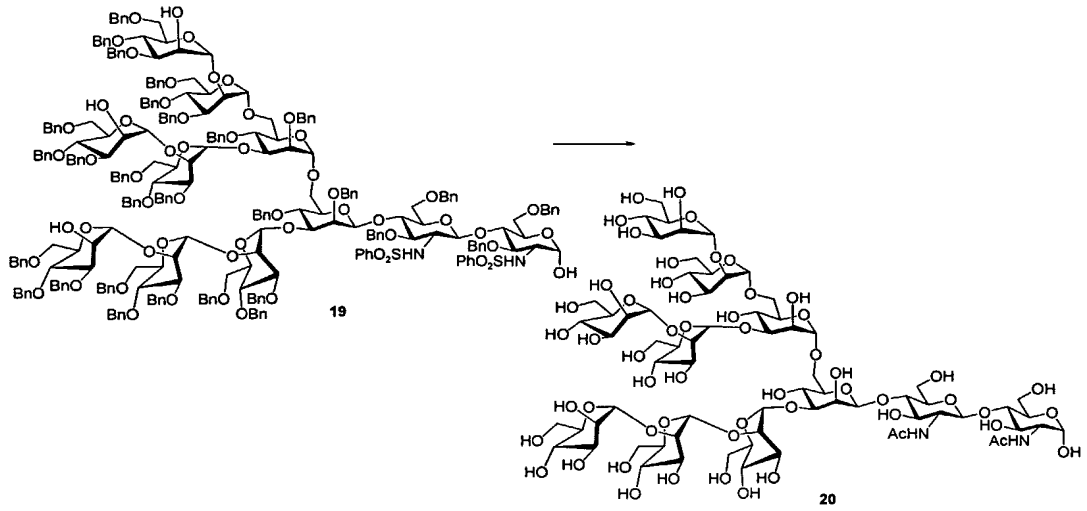


[0275] **Undecasaccharide triol 24**: **24** was prepared using the same procedure as described for **7**. **24**, white solide (468 mg, 96%). $[\alpha]_D^{25}$ 214.3 (*c* 0.23, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ 0.03 (s, 3 H), 0.05 (s, 3 H), 0.90 (s, 9 H), 5.07 (s, 1 H), 5.08 (s, 1 H), 5.13 (s, 1 H), 5.18 (s, 1 H), 5.21 (s, 1 H), 5.30 (s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -5.7, -4.5, 0.0, 14.1, 18.0, 21.0, 25.8, 29.6, 57.9, 58.6, 60.3, 67.6, 68.4, 68.6, 68.7, 68.9, 71.5, 71.9, 72.0, 72.3, 73.0, 73.1-73.5, 74.2, 74.5, 74.7, 74.9, 75.0, 75.2, 79.9, 80.0, 92.7, 99.4, 100.2, 100.7, 101.1, 101.5, 102.3, 126.7-128.7, 138.1-138.8, 140.7, 141.3. LRMS (ESI) calcd for $C_{287}H_{310}N_2O_{58}S_2SiNa_2$ $[M+2Na]^{2+}$ 2425.0, found 2425.2.

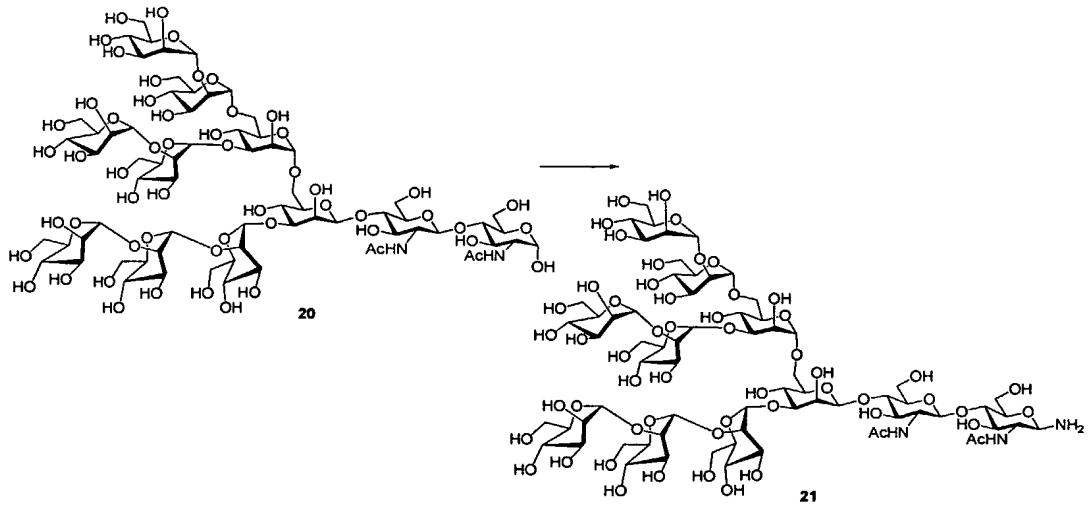


[0276] **Undecasaccharide tetraol 19**: To a solution of **24** (468 mg, 0.097 mmol) in HAc (1.0 M in THF, 2.5 mL) was added TBAF (1.0 M in THF, 2.5 mL) and the reaction mixture was stirred for 1 h before additional HAc (1.0 M in THF, 5.0 mL) was added. The mixture was concentrated and residue purified by column

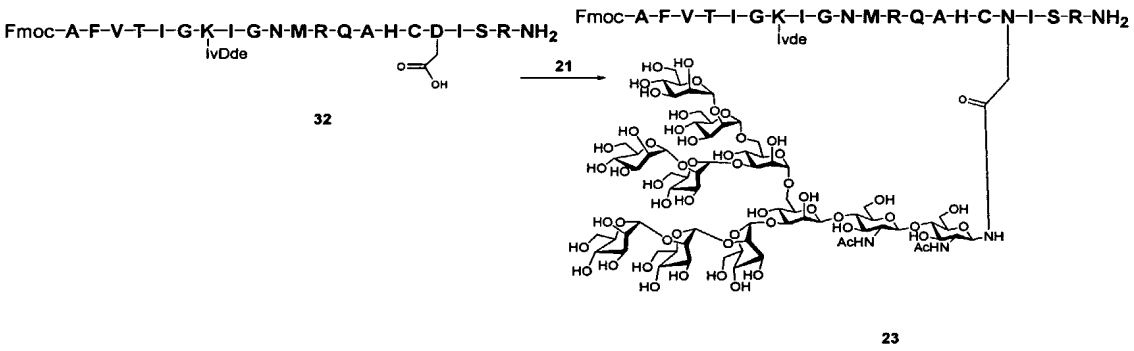
chromatography using 2.5% MeOH in CH_2Cl_2 as the eluent to afford **19** as a white solid (460 mg, 98%). $[\alpha]_D^{25}$ 121.7 (*c* 0.32, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ 4.95 (bs, 2 H), 5.08 (s, 1 H), 5.20 (bs, 2 H), 5.24 (s, 1 H), 5.27 (s, 1 H). LRMS (ESI) calcd for $C_{281}H_{296}N_2O_{58}S_2Na_2$ $[M+2Na]^{2+}$ 2367.9, found 2367.6.



[0277] **Glycan 20:** To a solution of sodium (101 mg, 4.391 mmol) in 15 mL liquid ammonia was added **19** (95 mg, 0.020 mmol) in THF (4 mL) at -78 °C and the reaction mixture was stirred for 2 h at -78 °C. The reaction was quenched with solid NH_4Cl at -78 °C and then warmed up to r.t. while argon was blowing through the reaction flask to evaporate all liquid. The residue was dried on vaccum for 2 h and dissolved in saturated $NaHCO_3$ aqueous solution (2 mL) and cooled to 0 °C. Ac_2O (0.1 mL) was then added at 0 °C and the ice bath was then removed and 5 min later additional Ac_2O (0.05 mL) was added. 30 min later, low resolution mass spectrum showed reaction is complete. The reaction mixture was loaded on to a Bio-Gel P-2 column (BIO-RAD, catalog number 150-4134, molecular cutoff 2000) using water as the eluent to remove salt and small molecular weight compounds. The fraction containing desired material (illustrated by MassSpectrum) was conbined and lyophilized to afford glycan **20** as a white solid (33 mg, 87% from **19**). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ 5.07 (bs, 2 H), 5.08 (s, 1 H), 5.13 (s, 1 H), 5.33 (s, 1 H), 5.36 (s, 1 H), 5.40 (s, 1 H). LRMS (ESI) calcd for $C_{70}H_{118}N_2O_{56}Na^+$ $[M+Na]^+$ 1905.6, found 1905.6.



[0278] **Glycosylamine 21**: A solution of **20** (33 mg, 0.018 mmol), NH_4Cl (10 g) in 30 mL water was heated to 40 °C for 2 days and Mass spectrum indicated that reaction is complete. So the reaction mixture was frozen and lyophilized. The residue was dissolved in 20 mL water, frozen and lyophilized again. This process was repeated until the weight of the residue is constant (36 mg). LRMS (ESI) calcd for $C_{70}H_{119}N_3O_{55}Na^+$ $[M+Na]^+$ 1904.7, found 1904.8.



[0279] **Gp120 glycopeptide 23**: A solution of peptide acid **32** (21 mg, 0.008 mmol), HATU (6 mg, 0.016 mmol), diehtylpropylamine (DIEPA) (2 μL, 0.011 mmol) in DMSO (150 μL) was stirred for 5 min and transferred to the flask containing **21** (5 mg, 0.002 mmol) and the reaction mixture was stirred for 2 h. Additional DIEPA was added (0.6 μL at 4 h and 0.6 μL at 6 h). At 7 h, a mixture of hydrazine, piperidine and DMF (volume ratio: 5:15:85, 0.2 mL) was added and the reaction mixture was stirred for 5 min and TFA in water (10%, 0.55 mL) was added

and stirred for 30 min. The crude solution was purified by HPLC using a Varian C18-DYNAMAX-60 Å column. HPLC Conditions: 10%B to 50%B over 50 min, UV 214 nM (A: 0.05% TFA in water; B: 0.04% TFA in CH_3CN). Retention time: 19.8 min. The fraction containing **23** was lyophilized to give **23** as a white solid (1.7 mg, 16% from **20**). ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ 4.99 (s, 1 H), 5.16 (s, 1 H), 5.19 (s, 1 H), 5.25 (s, 1 H), 8.29 (s, 1 H). LRMS (ESI) calcd for $C_{164}H_{275}N_{35}O_{80}S_2Na_3$ $[M+3Na]^{3+}$ 1360.6, found 1360.7; calcd for $C_{164}H_{275}N_{35}O_{80}S_2Na_4$ $[M+4Na]^{4+}$ 1020.7, found 1020.6.

[0280] **References:**

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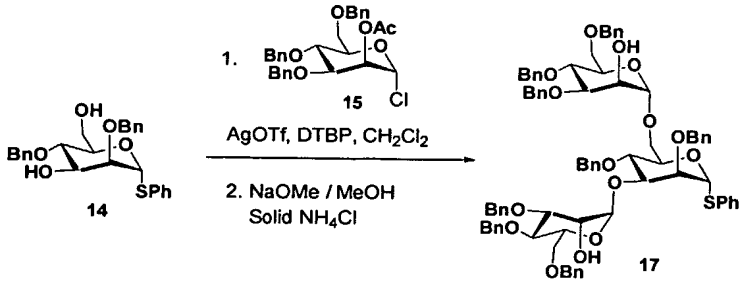
[0282] (2) Zhang, Y.-M.; Mallet, J.-M.; Sinay, P. *Carbohydrate Research* **1992**, *236*, 73-88.

[0283] (3) Marra, A.; Mallet, J. M.; Amatore, C.; Sinay, P. *Synlett* **1990**, 572-574.

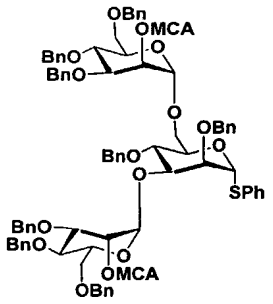
[0284] (4) Matsuo, I.; Wada, M.; Manabe, S.; Yamaguchi, Y.; Otake, K.; Kato, K.; Ito, Y. *Journal of the American Chemical Society* **2003**, *125*, 3402-3403.

[0285] (5) Calarese, D. A.; Scanlan, C. N.; Zwick, M. B.; Deechongkit, S.; Mimura, Y.; Kunert, R.; Zhu, P.; Wormald, M. R.; Stanfield, R. L.; Roux, K. H.; Kelly, J. W.; Rudd, P. M.; Dwek, R. A.; Katinger, H.; Burton, D. R.; Wilson, I. A. *Science (Washington, DC, United States)* **2003**, *300*, 2065-2071.

[0286] (6) Likhosherstov, L. M.; Novikova, O. S.; Derevitskaya, V. A.; Kochetkov, N. K. *Carbohydrate Research* **1986**, *146*, C1-C5.

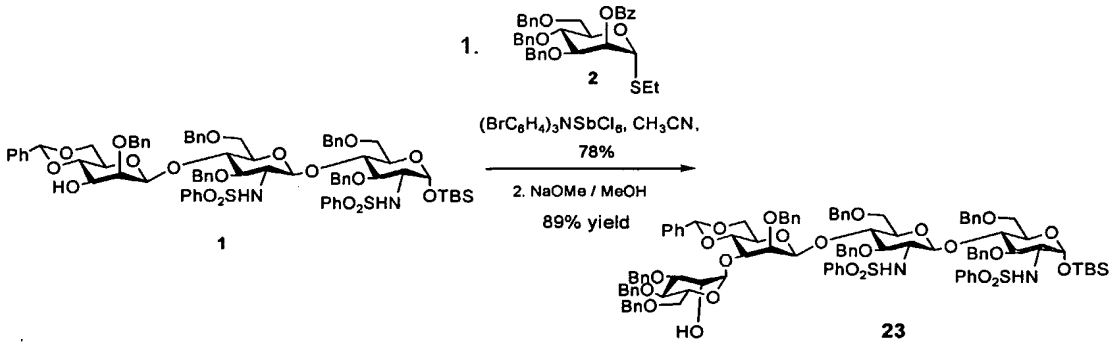


[0287] Into a 25 mL flask containing donor **15** (0.169 gm, 0.332 mmol) and acceptors **14** (0.037 gm, 0.083 mmol) (azeotropically dried with toluene) in 1.5 dichloromethane was added activated MS 4A and the mixture was stirred for 1 hr at room temperature. In a separate flask, AgOTf (0.087 gm, 0.332 mmol) and DTBP (0.078 mL, 0.347 mmol) in 1.5 mL of dichloromethane were stirred with MS 4A. After stirring for 1 hr, the flask containing the AgOTf / DTBP was cooled to – 10 °C and the solution containing mixture of donor and acceptor was added over 5 minutes. The solution was stirred in dark with gradual warming up to room temperature over 24 hr. The reaction mixture was diluted with ethyl acetate and was added aqueous saturated $NaHCO_3$. After stirring for 10 minutes, the reaction mixture was filtered through bed of Celite and the filtrate was washed with water, then with brine, dried over $MgSO_4$ and evaporated *in vacuo*. The crude product was purified by silica gel column chromatography (10% ethyl acetate / toluene) to afford semi pure trimer diacetate. This diacetate was dried azeotropically with toluene and dissolved in 2 mL of anhydrous methanol under argon. Sodium methoxide, 25% by weight in methanol (100 μL) was added and the reaction mixture was stirred for 12h. Solid ammonium chloride was added and the resulting solution was stirred for 20 min. The reaction mixture was carefully evaporated to solid residues, and the solid residues were washed with ethyl acetate. Evaporation of ethyl acetate layer provided crude product, which was purified by silica gel column chromatography (10% ethyl acetate / dichloromethane) to yield diol **17** in 65% yield (over two steps. [α] + 53.1 (c 1, $CHCl_3$); ^1H – NMR ($CDCl_3$, 400 MHz) δ 7.33-7.03 (45H, m, aromatic), 5.44 (1H, br-s), 5.17 (1H, br-s), 4.94 (1H, br-s), ; ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 138.68, 138.66, 138.4, 138.1, 138.06, 138.03, 134.9, 131.0, 129.3, 128.72, 128.70, 128.67, 128.61, 128.49, 128.47, 128.16, 128.13, 128.10, 128.01, 127.96, 127.88, 127.86, 127.83, 127.82, 127.79, 127.76, 127.71, 127.35, 99.9, 85.3, 80.5, 80.2, 79.6, 75.3, 75.15, 75.07, 74.6, 74.4, 73.8, 73.5, 72.7, 72.3, 72.2, 71.8, 71.7, 71.3, 69.5, 68.94, 68.90, 68.2, 66.4. ESI-MS calcd for $C_{80}H_{84}O_{15}S$ Na $[M+Na]^{1+}$ m/z = 1339.5: found 1339.5

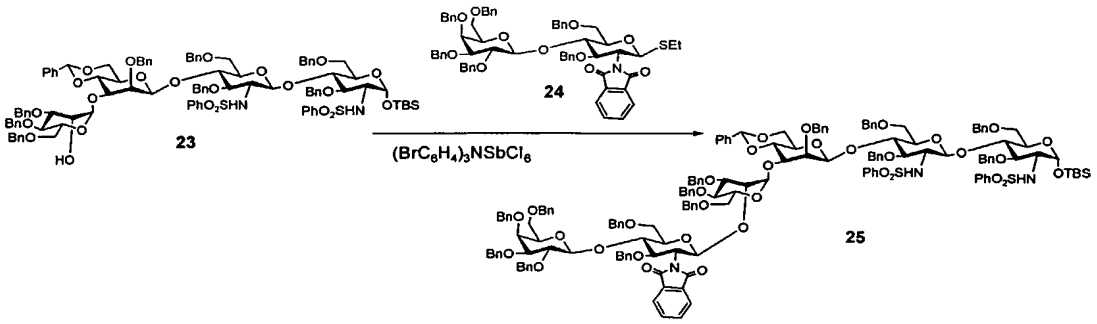


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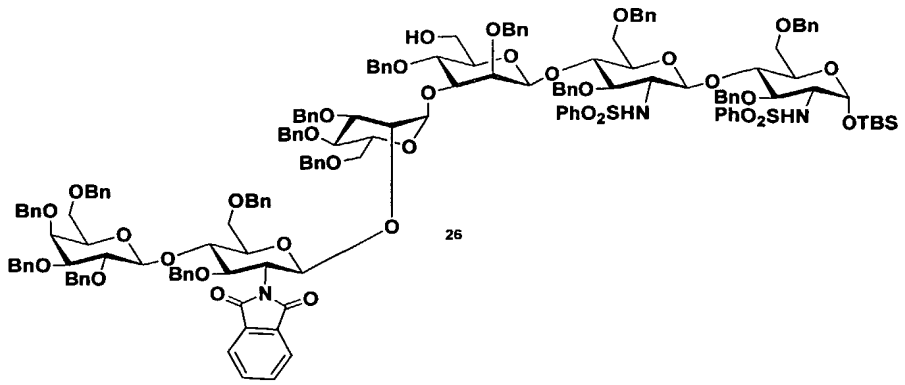
[0288] Into a 25 mL flask containing **17** (0.099 gm, 0.0689 mmol, azeotropically dried with toluene) in 0.4 mL mL of dry dichloromethane under argon and cooled to 0 °C. Pyridine (55 μL, mmol, 6.8 mmol) and chloroacetic anhydride (0.047 gm, .0275 mmol), were added successively and resulting reaction mixture was stirred for 2 h at 0 °C, and then diluted with ethyl acetate, washed two times with 0.5N HCl, water, sat $NaHCO_3$, brine, and dried with $MgSO_4$. Evaporation of ethyl acetate layer followed by silica gel column chromatography (20 ethyl acetate in hexanes) provided 0.166 gm (79% yield) of **22**. R_f 0.33 (20% ethyl acetate in hexanes). [α] + 58.2 (c 1, $CHCl_3$); ^1H – NMR ($CDCl_3$, 400 MHz) δ 7.30-7.03 (45H, m, aromatic), 5.47 (2H, m), 5.41 (1H, br-s), 5.15 (1H, s), 4.88 (1H, s), 4.75 (2H, t, *J* = 10.5 Hz); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 166.88, 166.78, 138.57, 138.51, 138.3, 137.79, 137.76, 137.74, 134.7, 130.9, 129.3, 128.7, 128.6, 128.59, 128.51, 128.48, 128.43, 128.30, 128.10, 128.0, 127.97, 128.86, 127.78, 127.75, 127.74, 127.7, 127.4, 99.5, 97.9, 84.9, 79.0, 78.1, 77.7, 75.4, 75.2, 75.1, 74.3, 74.1, 73.7, 73.5, 72.43, 72.37, 72.29, 71.78, 71.69, 70.7, 70.4, 69.0, 68.7, 66.8, 41.2, 41.0



[0289] Into a 25 mL flask containing donor **2** (125 mg, 0.0696 mmol, azeotropically dried with toluene) and 4A molecular sieves in dry acetonitrile were stirred for 1 hr under argon. Tris (4-bromophenyl) aminium hexachloroantimonate [$(BrC_6H_4)_3NSbCl_6$] (140 mg, promoter) and then a solution of acceptor **1** (100 mg, 0.0696 mmol) were added slowly while cooling the flask at 15 °C. After stirring for 15 min, another portion of tris (4-bromophenyl) aminium hexachloroantimonate [$(BrC_6H_4)_3NSbCl_6$] (46 mg) was added and the reaction mixture was warmed to room temperature and stirred for 3 hr. Freshly distilled triethyl amine (1.5 mL) was added to neutralize the reaction. The reaction mixture was filtered through a bed of Celite and concentrated. The crude product was purified by silica gel column chromatography to afford tetrasaccharide (0.110 gm). R_f 0.65 (20% ethyl acetate in toluene). Under argon this material was dissolved in mixture of dry methanol (2 mL) and dichloromethane (1.5 mL). Sodium methoxide, 25% by weight in methanol (0.038 mL) was added and stirred for 12 hr. Solid ammonium chloride was added and the mixture was evaporated to dryness. The solid residue was washed several times with ethyl acetate and concentrated. Purification by silica gel column chromatography afforded the 0.092 gm (89% yield) of **23**. R_f 0.42 (40% ethyl acetate in hexanes). [α] – 8.8 (c 1, $CHCl_3$);^{1}H – NMR ($CDCl_3$, 400 MHz) (selected signals) δ 7.75 (2H, d, J = 7.6 Hz), 7.72 (2H, d, J = 6.8 Hz), 5.41 (1H, br-s), 5.26 (1H, d, J = 2.0 Hz), 5.11 (1H, d, J = 2.4 Hz), 3.14 (1H, m), 3.0 (2H, m), 1.57 (1H, br-s), 0.908 (9H, s), 0.09 (3H, s), 0.03 (3H, s); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 141.7, 140.9, 138.7, 138.65, 138.61, 138.4, 138.0, 137.9, 137.8, 137.5, 132.6, 132.4, 129.5, 129.1, 129.0, (128.9-127.6), 127.4, 127.3, 127.2, 126.2, 101.5, 101.3, 101.1, 100.4, 93.0, 80.3, 80.0, 76.2, 75.8, 75.6, 75.5, 75.2, 74.8, 74.4, 73.96, 73.87, 73.6, 72.2, 72.0, 69.9, 69.3, 68.8, 68.5, 68.2, 67.8, 67.2, 37.5, 33.8, 33.6, 32.1, 30.3, 30.2, 29.9, 29.5, 29.1, 27.3, 26.9, 26.0, 23.4, 22.9, 19.9, 18.2, 14.4, 14.3, 7.6, - 4.2, - 5.4;

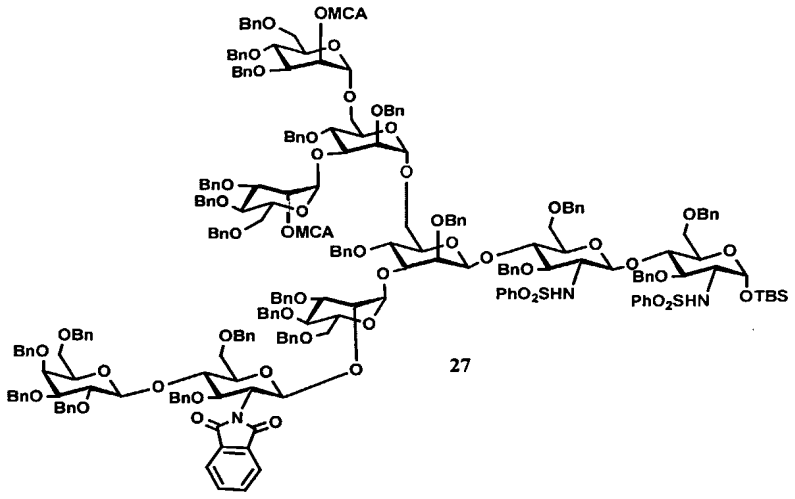


[0290] Into a 25 mL round-bottomed flask containing acceptor **23** (0.100 gm, 0.0535 mmol, azeotropically dried with toluene) in 1 mL dry acetonitrile was added 4A activated molecular sieves and was stirred at room temperature under argon for 1 hr. Similarly the donor **24** (0.1498 mmol, 0.158 gm) and 4A molecular sieves in 1 mL dry acetronitrile were stirred at room temperature for 1 hr. The flask containing donor was cooled to 15 °C and 0.091 gm of tris (4-bromophenyl) aminium hexachloroantimonate [$(BrC_6H_4)_3NSbCl_6$] (promoter) was added followed by the solution of acceptor. The reaction mixture was stirred at 15 °C for 20 min and then additional 0.031 gm of promoter was added. The cooling bath was removed and the reaction mixture was stirred for 3 hr. The reaction mixture was cooled to 0 °C and triethyl amine (1 mL) was added. After stirring 10 min at 0 °C, the reaction mixture was warmed to room temperature and stirred for additional 10 min. Filtering through a pad of Celite and evaporation of the filtrate afforded the crude product, which was purified by preparative TLC (first using 5% ethyl acetate in dichloromethane, then 30% ethyl acetate in hexanes) to afford .091 gm of **25** (60% yield), [α] – 16.6 (c 1, $CHCl_3$); ^1H – NMR ($CDCl_3$, 400 MHz) (selected signals) δ 7.72 (2H, d, J = 7.2 Hz), 7.67 (2H, d, J = 7.2 Hz), 5.21 (1H, br-s), 5.07 (1H, br-s), 5.01 (1H, br-s), 4.98 (1H, d, J = 8Hz), 2.99 (1H, m), 2.89 (1H, t, J = 8.4 Hz), 2.80 (1H, m), 2.64 (2H, m), 0.875 (9H, s), 0.07 (3H, s), 0.05 (3H, s); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 140.5, 139.7, 138.04, 138.01, 137.7, 137.69, 137.63, 137.59, 137.56, 137.47, 137.41, 137.3, 137.0, 136.8, 136.5, 136.4, 132.4, 131.3, 131.1, 130.9, 128.4, 128.1, 127.8, (127.5-126.1), 125.8, 125.3, 122.4, 122.0, 102.3, 100.3, 99.9, 99.4, 96.5, 94.7, 91.8, 81.5, 78.9, 77.6, 77.36, 77.31, 75.1, 74.4, 74.2, 74.1, 73.9, 73.8, 73.6, 73.5, 73.1, 72.8, 72.7, 72.4, 72.2, 71.9, 71.8, 71.6, 71.5, 71.2, 57.8, 56.9, 51.3, 28.7, 24.8, 16.9, 7.6, - 5.4, - 6.6



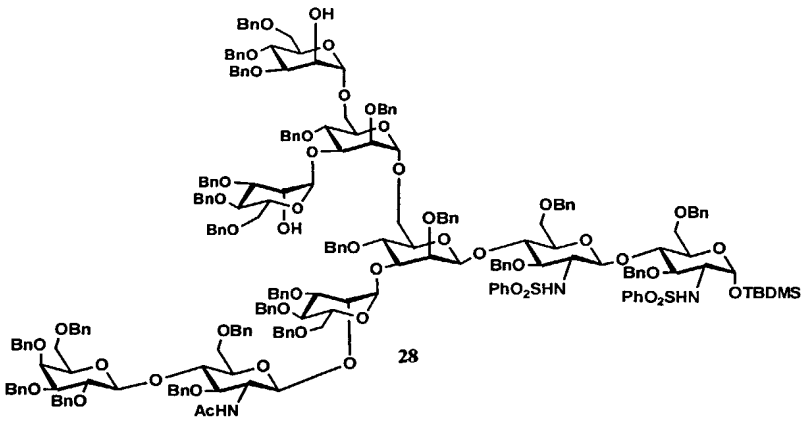
[0291] To the azeotropically dried **25** (0.307 gm, 0.107 mmol) in 25 mL round bottomed flask was added 8 mL of BH_3.THF (1 molar) and stirred for 5 minutes at room temperature. The reaction mixture was cooled to 0 °C and 0.35 mL of $n$$Bu_2OTf$ (1 molar in CH_2Cl_2) was added. The resulting reaction mixture was stirred at 0 °C for 9 hr. Freshly distilled triethyl amine (0.492 mL) was added and followed by careful addition of methanol until the evolution of H_2 had ceased. The reaction was evaporated to dryness, twice codistilled from methanol to afford the crude product as clear oil. Purification by silica gel column chromatography (30% ethyl acetate in hexanes) provided the **26** in 75% yield (0.231 gm).

[0292] [α] – 7.0 (c 1, $CHCl_3$); ^1H – NMR ($CDCl_3$, 400 MHz) (selected signals) δ 7.74 (2H, d, J = 7.2 Hz), 7.69 (2H, d, J = 6.8 Hz), 5.07 (2H, m), 2.96 (1H, m), 2.75 (2H, m), 0.90 (9H, s), 0.07 (3H, s), 0.02 (3H, s); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 168.5, 167.7, 141.4, 140.9, 139.3, 139.2, 138.97, 138.91, 138.73, 138.66, 138.64, 138.58, 138.50, 138.45, 138.27, 138.1, 137.8, (129.2-126.9), 123.5, 123.4, 103.3, 101.0, 100.9, 99.2, 96.5, 92.9, 82.6, 80.1, 79.9, 78.7, 78.4, 76.5, 76.0, 75.9, 75.4, 75.1, 74.9, 74.8, 74.68, 74.63, 74.49, 74.34, 74.29, 74.24, 73.84, 73.77, 73.6, 73.45, 73.3, 73.2, 73.0, 72.8, 72.7, 61.4, 60.6, 58.6, 59.2, 55.8, 26.0, 21.3, 19.3, 18.2, 14.4, 14.1, - 4.2, - 5.4;



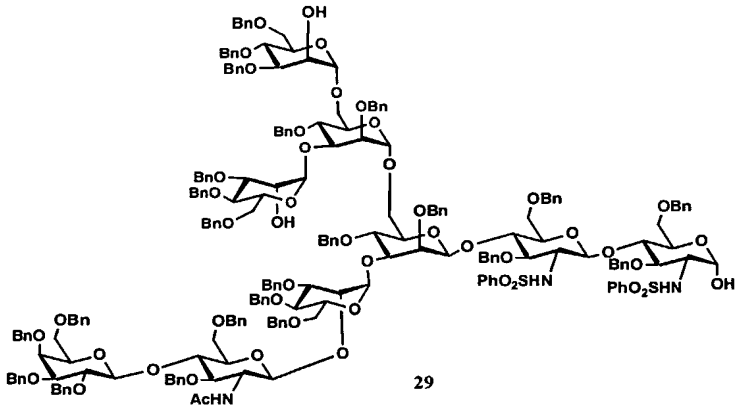
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[0293] Into a 5 mL v vial were taken azeotropically dried donor **22** and acceptor in **26** mL anhydrous acetonitrile and activated 4A MS was added. The resulting reaction mixture was stirred under argon for 1 hour at room temperature and then was cooled to 15 °C. At this point Tris (4-bromophenyl) aminium hexachloroantimonate [$(BrC_6H_4)_3NSbCl_6$] was added to the reaction mixture. The cooling bath was removed and the reaction mixture was stirred at room temperature for 12 h or TLC indicated the disappearance of the acceptor. The reaction mixture was cooled to 0 °C and 2 mL triethyl amine was added and stirred for 30 minutes with gradual warming up to room temperature. The reaction mixture was filtered through a pad of Celite and concentrated to provide crude material, which was purified by preparative TLC (20x20cm x 1 mm thickness PK6F plates) using 40% ethyl acetate in hexanes to yield **27**. [α] + 9.4 (c 1, $CHCl_3$); ^1H – NMR ($CDCl_3$, 400 MHz) (selected protons) δ 5.41 (1H, br-s), 5.32 (1H, br-s), 5.09 (1H, br-s), 4.97 (2H, m), 0.83 (9H, s), 0.05 (s, 3H), 0.03 (s, 3H); ^{13}C-NMR ($CDCl_3$, 100 MHz)□ 168.6, 167.6, 166.70, 166.67, 141.5, 140.9, 139.30, 139.23, 139.0, 138.9, (138.8-138.1), 137.89, 137.86, 137.6, 133.6, 132.5, 132.3, 132.0, (129.0-126.9), 126.7, 103.3, 101.7, 100.9, 99.3, 98.0, 97.8, 96.3, 92.9, 82.6, 81.3, (78.9-65.1), 58.6, 58.1, 55.8, 39.9, 39.8, 28.7, 24.8, 17.0, - 5.4, - 6.7.

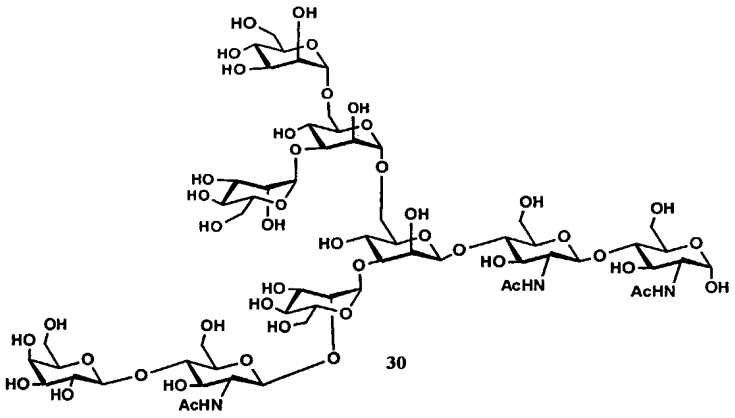


[0294] To azeotropically dried **27** (100 mg, 0.023 mmol) in a v 5 mL v vial equipped with spin bar were added fresh toluene (2 mL) and *n*-butanol (4 mL). Ethylenediamine was added and the reaction mixture was heated at 90 o C for 18 hr. After cooling to room temperature, the reaction mixture was concentrated under *vacuo*. The crude product was dissolved in 5 mL of toluene and evaporated to dryness. Pyridine and acetic anhydride were added and the reaction mixture was stirred for 16 hr at room temperature. The reaction mixture was evaporated to dryness, twice from toluene, yielding foam with some solid. This material was dissolved in 5 mL of methanol and 2 mL of THF under argon and 0.35 mL of 25% sodium methoxide in methanol was added and the reaction mixture was stirred for 12 hr. Solid ammonium chloride was added and stirred for 30 min. Careful evaporation of this biphasic reaction mixture provided white solid residue, which was washed three times by ethyl acetate. Concentration of ethyl acetate layer yielded the crude product, which was purified by preparative TLC (10% ethanol in toluene) to afford **28** in 69% yield (0.064 gm). R_f = 0.67 (10 ethanol in toluene). [α] + 14.6 (c 1, $CHCl_3$); ^1H – NMR ($CDCl_3$, 500 MHz) (selected protons) δ 7.65 (2H, d, *J* = 9 Hz), 7.60 (2H, d, *J* = 7.5 Hz), 5.22 (1H, d, *J* = 8 Hz), 5.10 (1H, br-s), 5.07 (2H, br-s), 3.06 (1H, m), 2.96 (1H, m), 2.24 (2H, d, *J* = 14.5 Hz), 1.68 (3H, s), 0.90 (9H, s), 0.07 (3H, s), 0.027 (3H, s). ^{13}C-NMR ($CDCl_3$, 125 MHz)δ 169.6, 141.5, 140.9, 139.6, 139.3, 139.1, 138.97, 138.95, 138.89, 138.7, 138.6, 138.5, 138.39, 138.36, 138.3, 138.2, 138.0, 137.7, 132.6, 132.4, 129.0, 128.9, (128.7-127.3), 127.2, 126.7, 103.0, 102.0, 100.9, 100.0, 99.9, 98.3, 97.793.0, 82.5, 81.4, 80.2, 80.1, 79.7, 79.4, 78.8, 78.6, 78.1, 77.9, 77.8, 76.6, 76.0, 75.3, 75.2, 74.9, 74.8, 74.7, 74.4, 74.38,

74.35, 74.2, 73.9, 73.7, 73.6, 73.5, 73.49, 73.45, 73.37, 73.2, 73.1, 72.0, 71.9, 71.8, 71.4, 71.4, 71.3, 71.1, 70.0, 69.7, 69.1, 68.9, 68.7, 68.4, 67.9, 67.8, 66.7, 65.7, 58.8, 58.2, 57.2, 26.0, 23.6, 18.2, 1.2, -4.2, 5.4.

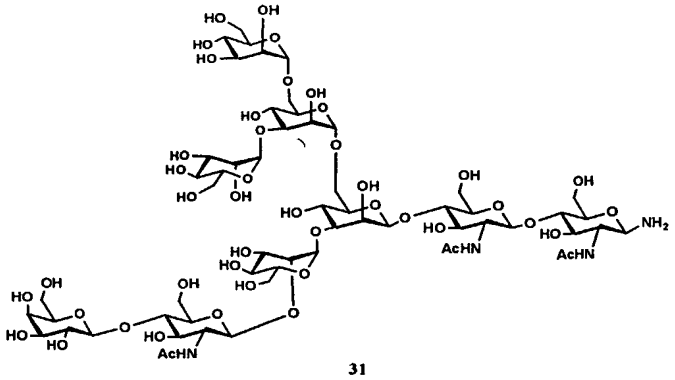


[0295] To the azeotropically dried **28** in a 25 mL round bottomed flask equipped with stir bar was added 0.5 mL 1M acetic acid in THF and the reaction mixture was cooled to 0 °C. To this ice cooled reaction mixture was added 0.5 mL TBAF (1M in THF). The cooling bath was removed and the reaction mixture was stirred for 3 hr. Additional 2 mL 1M acetic acid in THF was added and the reaction mixture was stirred for 15 min. The reaction mixture was evaporated to dryness and the crude product was purified by preparative TLC (10% ethanol in toluene) to afford 0.055 gm (89% yield)of **29**. [α] + 9.40 (c 1, $CHCl_3$); ^1H – NMR ($CDCl_3$, 500 MHz) (selected protons) δ 7.66 (2H, d, J = 8 Hz), 7.60 (2H, d, J = 7.6 Hz), 5.17 (1H, d, J = 7.2 Hz), 5.07 (1H, br-s), 5.01 (2H, m), 2.34 (1H, br), 2.18 (1H, br), 1.61 (s, 3H),



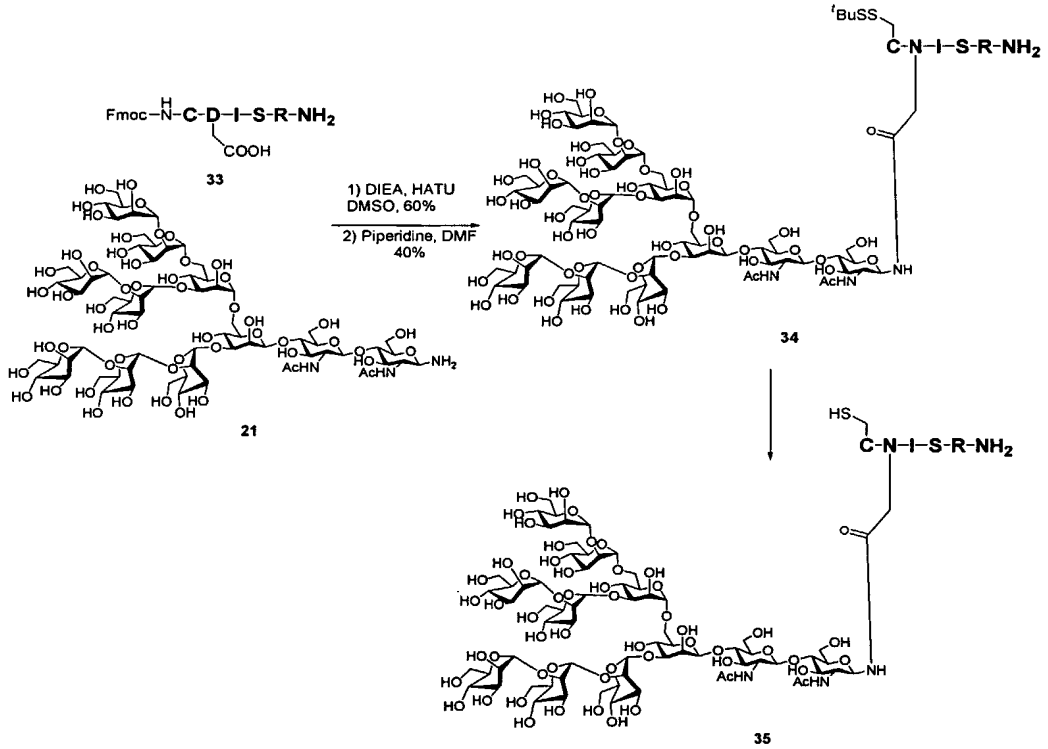
30

[0296] Into a three necked round bottomed flask, equipped with dry ice acetone condenser was condensed 15 mL ammonia under argon. Sodium metal (0.095 gm, 153 equiv.) was added in three portions. The resulting blue solution was stirred for 30 min at – 78 °C. The compound **29** (0.104 gm, 0.027 mmol) in 1.5 mL (3 x 0.5 mL) was added to the solution and the reaction mixture was stirred for 2 hr. Solid ammonium chloride (0.263 gm, 4.97 mmol) was added to quench the reaction and the reaction mixture was warmed to room temperature slowly. Evaporation of the residual liquid provided solid residue, which was dissolved in 5 mL pyridine. To this mixture was added acetic anhydride (3 mL) and DMAP (5 mg) and the resulting mixture was stirred with slowly warming to room temperature over 12 hr. The reaction mixture was evaporated to dryness and purified carefully by silica gel column chromatography to afford peracetate. The peracetate in 5 mL methanol was added solution of NaOMe, 25% by weight in methanol (0.4 mL) and was stirred for 24 hr. The resulting cloudy solution was treated with water at 0 °C and stirred for another 24 hr. The reaction mixture was neutralized using Amberlyst – 15 acidic regin and evaporated to afford crude product, which was purified by size exclusion chromatography using Bio-Gel P2 regin yielding 30 mg of free glycan.



31

[0297] Free glycan (10 mg) in 15 mL of saturated ammonium bicarbonate was heated at 40 °C. Additional ammoniumhydrogen carbonate was added time to time to keep the solution saturated. After two days of stirring the content of the flask was shell frozen, lyophilized, dissolved in water (10 mL), lyophilized; this process was repeated until the white solid residue reached constant mass of 10 mg, which was used directly in the next step.

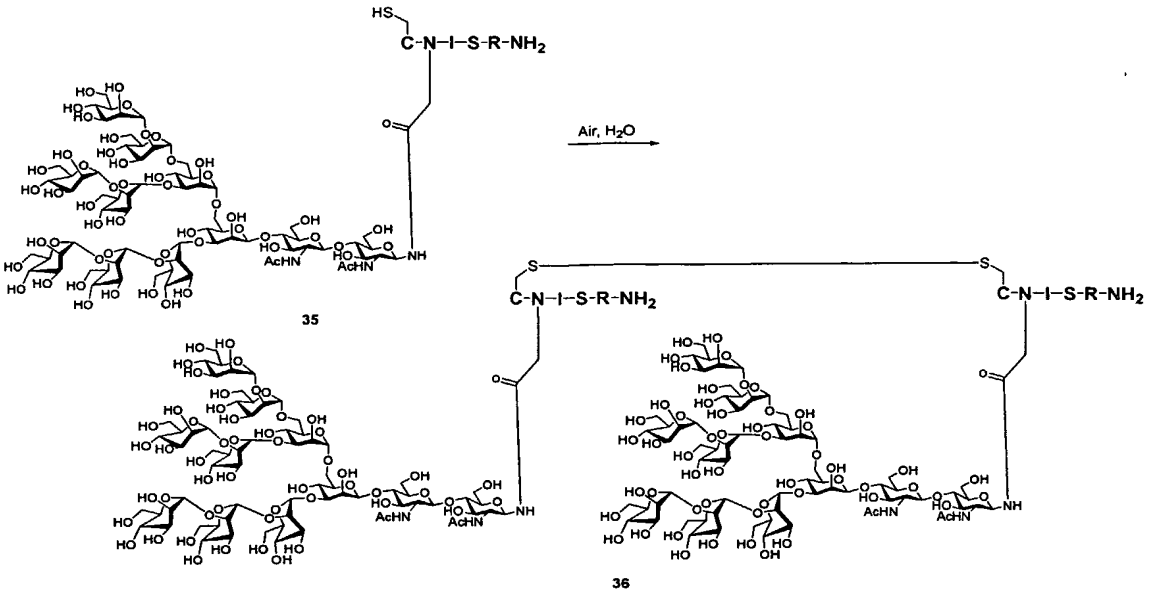


[0298] **Glycopeptide 34:**

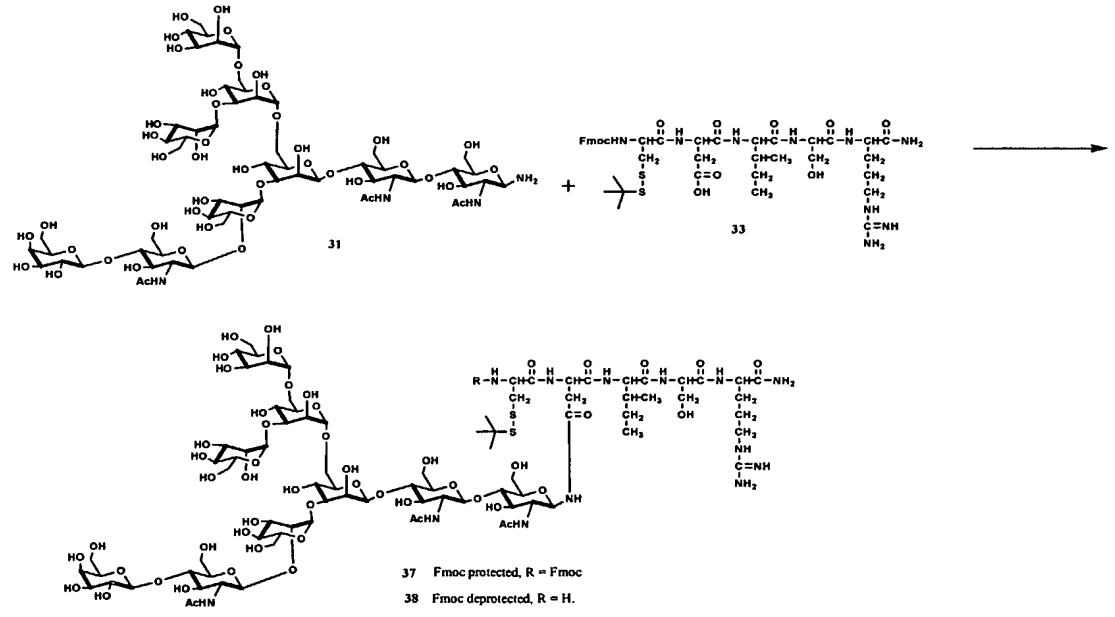
[0299] A solution of acid **33** (6 mg, 0.007 mmol), HATU (5 mg, 0.013 mmol), DIEPA (1.7 μL, 0.012 mmol) in DMSO (0.1 mL) was stirred for 10 min and transferred to a falcon tube (25 mL) containing 4.2 mg of **21**. The solution was stirred for 2 h and additional DIEPA (1.2 μL) was added. The reaction mixture was purified by semiprep HPLC column (30 to 50%B over 20 min) to afford Fmoc-protected glycopeptide (3.6 mg, 60%). LRMS (ESI) calcd for $C_{111}H_{177}N_{12}O_{64}S_2Na^{++}$ $[M+H+Na]^{++}$ 1394.5, found 1394.5. This Fmoc-protected glycopeptide was dissolved in 0.4 mL pipyridine/DMF (1:4) solution and stirred for 15 min and quenched by THF/H_2O (10%) until the pH = 2~3. The crude mixture was purified on semiprep HPLC column (5 to 25%B over 20 min) to afford **34** (2 mg, 40%). LRMS (ESI) calcd for $C_{96}H_{167}N_{12}O_{62}S_2Na^{++}$ $[M+H+Na]^{++}$ 1283.5, found 1283.6. ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ 4.99 (s, 1 H), 5.02 (s, 1 H), 5.16 (s, 1 H), 5.18 (s, 1 H), 5.25 (s, 1 H).

[0300] **Glycopeptide 35:**

[0301] To a solution of **34** (2 mg, 0.0008 mmol) in phosphorous buffer (NaH_2PO_4 and Na_2HPO_4, pH=7.4, 0.5 mL) was added $HSCH_2CH_2SO_3Na$ (10 mg, 0.061 mmol) and stirred for 2 days. TCEP (30 mg, 0.104 mmol) was then added and the resulting solution was stirred for 1 h. The residue was purified on semiprep HPLC column (5 to 25%B over 20 min) to afford **35** (1.7 mg, 60%). LRMS (ESI) calcd for $C_{92}H_{160}N_{12}O_{62}S^{++}$ $[M+2H]^{++}$ 1228.5, found 1228.5. ^1H NMR (400 MHz, $CDCl_3$) selected signals: δ 4.90 (s, 1 H), 4.99 (s, 1 H), 5.15 (s, 1 H), 5.18 (s, 1 H), 5.25 (s, 1 H).

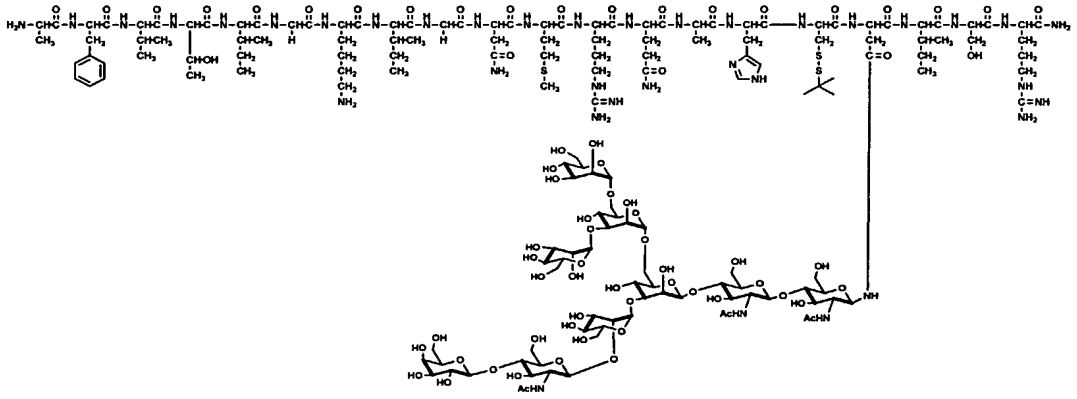


Glycopeptide, when dissolved in H_2O and exposed to air, formed dimmer **36**. HPLC (Varian Microsorb 100-5-C18) retention time, 12.75 min (0% to 40% acetonitrile in water). LRMS: (ESI) $C_{184}H_{317}N_{24}O_{124}S_2$: $[M+3H]^{+++}$: calculated: 1636.95, found, 1636.99.



[0302] To a 15 mL polypropylene conical tube equipped with stir bar in 0.2 mL DMSO was added peptide **33** (11 mg, 3 equiv.) and HATU (15 mg, 5.9 equiv.). The solution was stirred for 1 min and was added diisopropyl ethyl amine (3.58 μL,

3 equiv.) and was stirred for another minute. This orange-yellow solution was transferred *via* 0.5 mL syringe to the flask containing glycosylamine **31** (11 mg). The conical tube was rinsed with additional 0.1 mL of DMSO and transferred to the flask containing glycosyl amine using the same 0.5 mL syringe. Monitoring by LCMS showed that no additional product formation after 6 hr. Purification of the reaction mixture by size exclusion chromatography provided the **37**. To this Fmoc protected **38** was added a 1 : 3 : 16 mixture of hydrazine : piperidine : DMF (200 μL). The resulting yellowish solution was stirred for 30 min before addition of a solution of TFA to bring the pH to 3. The reaction mixture was purified by semiprep HPLC column (5 to 25%B over 25 min) to afford the Fmoc deprotected **38** in 30% yield. ^{1}H – NMR ($CDCl_3$, 500 MHz) (selected protons) δ 4.78 (2H, d, J = 12.4 Hz), 4.70 (1H, d, J = 9.6 Hz), 4.57 (2H, d, J = 13.2 Hz), 4.26 (4H, m), 4.14 (1H, d, J = 7.2 Hz), 1.01 (9H, s), 0.575 (6H, m).



39

[0303] Compound **39** was prepared similar to **34**.

ABBREVIATIONS AND GLOSSARY

[0304] A: alanine

[0305] Ac: acetyl

[0306] ACT: α1-antichymotrypsin

[0307] Ala: alanine

[0308] Arg: arginine

[0309] Asn: asparagine

[0310] Asp: aspartic acid

[0311] Bn: benzyl

[0312] Boc: *tert*-butyloxycarbonyl

[0313] BPH: benign prostatic hyperplasia

[0314] BSP: benzenesulfinyl piperidine

[0315] Bu: butyl

[0316] Bz: benzoyl

[0317] CAN: ceric ammonium nitrate

[0318] coll: *sym*-collidine

[0319] C-terminus: peptide carbonyl terminus

[0320] Cys: cysteine

[0321] D: aspartic acid

[0322] DIEA: *N,N*-diisopropylethylamine

[0323] DMF: dimethyl formamide

[0324] DMSO: dimethyl sulfoxide

[0325] DTBMP: di-*tert*-butylmethylpyridine

[0326] DTBP: di-*tert*-butylpyridine

[0327] Et: ethyl

[0328] Fmoc: 9-fluorenylmethyloxycarbonyl

[0329] G: glycine

[0330] Gal: galactose

[0331] Glc: glucose

[0332] Gln: glutamine

[0333] Glu: glutamic acid

[0334] Gly: glycine

[0335] H: histidine

[0336] HATU: 7-azahydroxybenzotriazolyl tetramethyluronium hexafluorophosphate

[0337] His: histidine

[0338] Ile: isoleucine

[0339] K: lysine

[0340] kDa: kilodaltons

[0341] KLH: keyhole limpet hemocyanin

[0342] L: leucine

[0343] Leu: leucine

[0344] Lys: lysine

[0345] Man: mannose

[0346] MES-Na: 2-mercaptoethanesulfonic acid, sodium salt

[0347] MHC: major histocompatibility complex

[0348] N: asparagine

[0349] NAc: *N*-acetyl

[0350] NCL: native chemical ligation

[0351] N-terminus: peptide amine terminus

[0352] *O*-linked: linked through an ethereal oxygen

[0353] Pam3Cys: tripalmitoyl-S-glycerylcysteinylserine

[0354] PBS: phosphate-buffered saline

[0355] Ph: phenyl

[0356] Phth: phthalimido-

[0357] PMB: *p*-methoxybenzyl

[0358] Pro: proline

[0359] Gp120: prostate specific antigen

[0360] Py: pyridine

[0361] QS21: a glycosteroidal immunoaduvant

[0362] R: arginine

[0363] S: serine

[0364] sat. aq.: saturated aqueous

[0365] Ser: serine

[0366] T: threonine

[0367] TBAF: : tetra-*n*-butylammonium fluoride

[0368] TBS: *tert*-butyldimethylsilyl

[0369] *t*Bu: *tert*-butyl

[0370] Tf: trifluoromethanesulfonate

[0371] THF: tetrahydrofuran

[0372] Thr: threonine

[0373] t-Gp120: total prostate specific antigen

[0374] Trp: tryptophan

[0375] V: valine

[0376] Val: valine

[0377] W: tryptophan

- APPENDIX A –

cross-linking

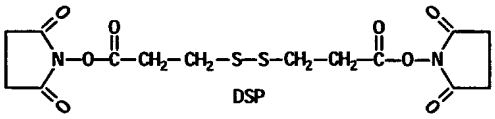
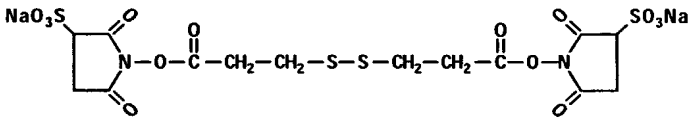
Applications for Use of Cross-linkers

Cell Surface Cross-linking

To ensure cell-surface specific cross-linking for identification of surface receptors or their ligands, it is best to use membrane-impermeable cross-linkers. In the past, researchers used water-insoluble cross-linkers and carefully controlled the amount of cross-linker and the cross-linking duration. This prevented penetration of the membrane by the cross-linker and subsequent reaction with membrane proteins. Many references cite the use of membrane-permeable cross-linkers for cell surface cross-linking. Staros developed water-soluble sulfo-NHS analogs as alternatives to membrane permeable, homobifunctional NHS-ester and imidoester cross-linkers.[26] The sulfo-NHS-ester, homobifunctional cross-linker BS_3 (Product #21579) is very useful for cell surface cross-linking of ligands to receptors through primary amines on each. The sulfonyl groups attached to the succinimidyl rings of sulfo-NHS cross-linkers make them membrane-impermeable and non-reactive with inner membrane proteins. Therefore, cross-linking time and quantity of cross-linker are less critical when using sulfo-NHS-esters. Pierce offers a variety of sulfo-NHS-ester cross-linkers, both homobifunctional and heterobifunctional. Homobifunctional sulfo-NHS-esters, heterobifunctional sulfo-NHS-esters and photoreactive phenyl azides are good choices for cross-linking on the surface of a cell. See Tables 3, 5 and 9 for specific characteristics and selection of cross-linkers for cell surface applications.

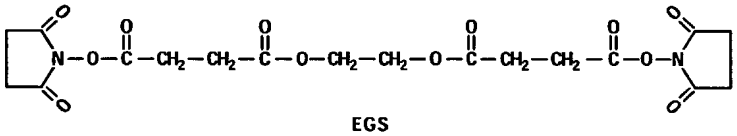
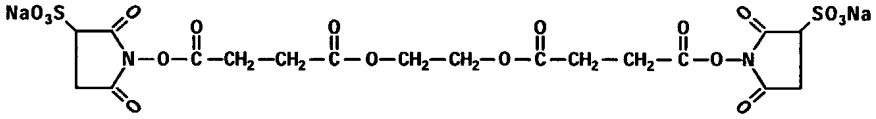
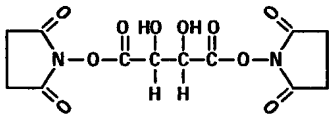
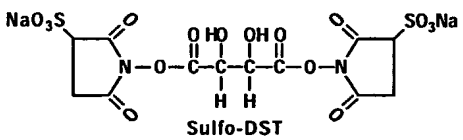
cross-linking

Table 3: Homobifunctional NHS-Ester Cross-linkers (Continued)

Cross-Linker	Product #	M.W.	Spacer Arm Length	Reactivity/ Characteristics	Applications/ References
DSP Dithiobis(succinimidyl propionate)  DSP	22585	404.42	12 Å	One of the most widely used cross-linkers, also known as Lomant's Reagent.[41] Water-insoluble, thiol-cleavable—can be cleaved with 10-50 mM DTT at 37°C for 30 minutes or with 5% ß-mercaptoethanol in SDS-PAGE sample buffer (2% SDS, 6.25 mM Tris base, 10% glycerol) at 100°C for 5 minutes.	• Examining spatial relationships of the capsid polypeptides of the mengo virion[91] • Studying renal Na+ and K+-ATPase[92] • Nearest neighbor relationships of bovine mitochondrial H+-ATP[93] • Producing interactions between protein components of the chemotaxis mechanism in E. coli[94] • Chemical cross-linking of a-CPI[95] • Identifying cross-linked cytochrome P-450 in rat liver microsomes[96] • Studying the influence of metal ions on prothrombin self-association[97] • Studying glycoprotein topology on intact human red blood cells[98] • Molecular identification of receptors for vasoactive intestinal peptide in rat intestinal epithelium[99] • Characterization of a cell surface receptor for colony-stim ulating factor (CSF-2a)[100] • Determining membrane antigens by covalent cross-linking to monoclonal antibodies[101]
DTSSP [3,3'-Dithiobis(sulfosuccinimidyl propionate)  DTSSP	21577	608.51	12 Å	Water-soluble analog of DSP[86]	• Cross-linking the extracytoplasmic domain of the anion exchange channel in intact human erythrocytes[88] • Cross-linking studies on Novikoff ascites hepatoma cytokeratin filaments[89] • Characterization of the B lymphocyte Fc receptor for IgE[90] • Cross-linking platelet glycoprotein Ib[102] • Characterization of a membrane-ribosome complex in B. subtilis[103]

cross-linking

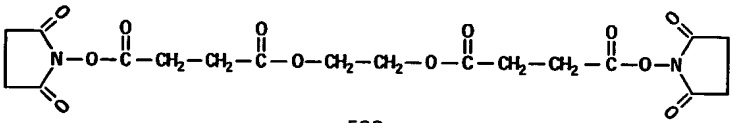
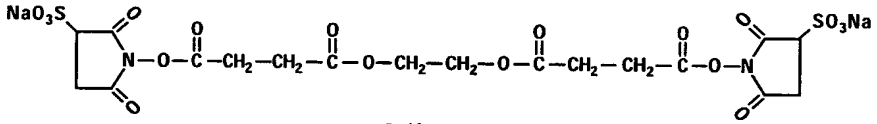
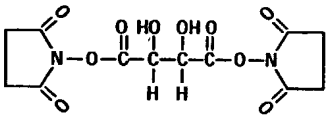
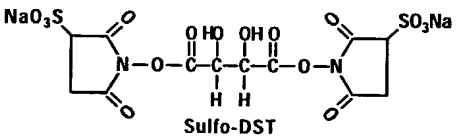
Table 3: Homobifunctional NHS-Ester Cross-linkers (Continued)

Cross-Linker	Product #	M.W.	Spacer Arm Length	Reactivity/ Characteristics	Applications/ References
EGS Ethylene glycobis (succinimidylsuccinate)  EGS	21565	456.37	16.1 Å	Water-insoluble hydroxylamine. Cleavable—cleaved by incubating with 1 M hydroxylamine for 3-6 hours at 37°C at pH 8.5. Lactose dehydrogenase retained 60% of its activity after cross-linking with EGS.[104]	• Cross-linking studies of cytochrome P-450 and reduced nicotinamide adenine dinucleotide phosphate-cytochrome P-450 reductase[105] • Tumor necrosis factor (TNF) and lymphotoxin LT cross-linking[106] • Converting a gonadotropin-releasing hormone antagonist to an agonist[107] • Preparing the EGS dimer of the GnRH agonist D-Lys-GnRH[108] • Covalent cross-linking of vasoactive peptide to its receptors on intact human lymphoblasts[76] • Binding and cross-linking of ^{125}I-gastrin releasing peptid (GRP)[109]
Sulfo-EGS Ethylene glycobis(sulfo-succinimidylsuccinate)  Sulfo-EGS	21566	660.47	16.1 Å	Water-soluble analog of EGS. Reactions similar to EGS.[104]	104
DST Disuccinimidyl tartarate  DST	20590	344.24	6.4 Å	Water-insoluble sample cross-linked with DST in first-dimensional gel. Cleavable by soaking in 0.015 M sodium periodate, 0.1% SDS, 0.02 M sodium phosphate, pH 7.0 for 2 hours (with several changes) at room temperature.[110]	• Cross-linking of ubiquinone cytochrome c reductase (complex III)[110] • Characterization of the cell surface receptor for colony-stimulating factor (CSF-2a)[111] • Cross-linking study of the Ca^{2+}, Mg^{2+} activated adenosine triphosphate of E. coli[100] • Human promyelocytic cell line cross-linking of cell lysate with DST[112]
Sulfo-DST Disulfosuccinimidyl tartarate  Sulfo-DST	20591	548.34	6.4 Å	Water-soluble analog of DST	100,110-112

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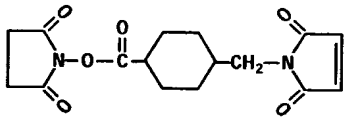
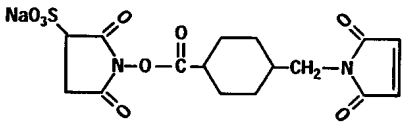
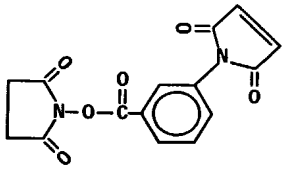
cross-linking

Table 3: Homobifunctional NHS-Ester Cross-linkers (Continued)

CROSS-LINKER	PRODUCT #	M.W.	SPACER ARM LENGTH	REACTIVITY/ CHARACTERISTICS	APPLICATIONS/ REFERENCES
EGS Ethylene glycobis (succinimidylsuccinate)  EGS	21565	456.37	16.1 Å	Water-insoluble hydroxylamine. Cleavable—cleaved by incubating with 1 M hydroxylamine for 3-6 hours at 37°C at pH 8.5. Lactose dehydrogenase retained 60% of its activity after cross-linking with EGS.[104]	• Cross-linking studies of cytochrome P-450 and reduced nicotinamide adenine dinucleotide phosphate-cytochrome P-450 reductase[105] • Tumor necrosis factor (TNF) and lymphotoxin LT cross-linking[106] • Converting a gonadotropin-releasing hormone antagonist to an agonist[107] • Preparing the EGS dimer of the GnRH agonist D-Lys-GnRH[108] • Covalent cross-linking of vasoactive peptide to its receptors on intact human lymphoblasts[76] • Binding and cross-linking of ^{125}I-gastrin releasing peptid (GRP)[109]
Sulfo-EGS Ethylene glycobis(sulfo-succinimidylsuccinate)  Sulfo-EGS	21566	660.47	16.1 Å	Water-soluble analog of EGS. Reactions similar to EGS.[104]	104
DST Disuccinimidyl tartarate  DST	20590	344.24	6.4 Å	Water-insoluble sample cross-linked with DST in first-dimensional gel. Cleavable by soaking in 0.015 M sodium periodate, 0.1% SDS, 0.02 M sodium phosphate, pH 7.0 for 2 hours (with several changes) at room temperature.[110]	• Cross-linking of ubiquinone cytochrome c reductase (complex III)[110] • Characterization of the cell surface receptor for colony-stimulating factor (CSF-2a)[111] • Cross-linking study of the Ca^{2+}, Mg^{2+} activated adenosine triphosphate of E. coli[100] • Human promyelocytic cell line cross-linking of cell lysate with DST[112]
Sulfo-DST Disulfosuccinimidyl tartarate  Sulfo-DST	20591	548.34	6.4 Å	Water-soluble analog of DST	100,110-112

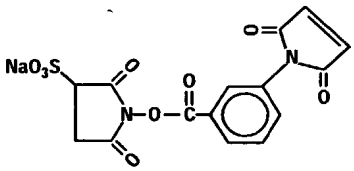
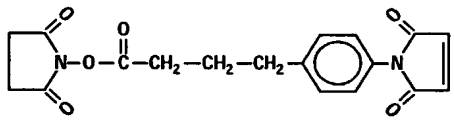
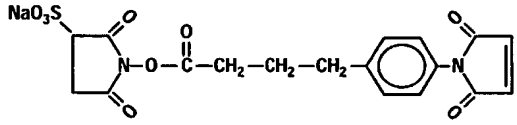
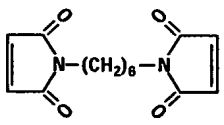
cross-linking

Table 5: NHS-Ester-Maleimide Heterobifunctional Cross-linkers

CROSS-LINKER	PRODUCT #	M.W.	SPACER ARM LENGTH	REACTIVITY/ CHARACTERISTICS	APPLICATIONS/ REFERENCES
SMCC Succinimidyl 4-(N-maleimido-methyl) cyclo-hexane-1-carboxylate  SMCC	22320	334.33	11.6 Å	Water-insoluble, noncleavable, very stable. Maleimide-reactive group.	• Conjugation of glucose oxidase from Aspergillus niger to rabbit antibodies[46] • Conjugating Fab' to horseradish peroxidase[116-119, 125] • Conjugating affinity-purified antidigoxin $F(ab')_2$ fragments to ß-galactosidase[120] • Enzyme labeling of antibodies and antibody fragments[121] • Conjugating alkaline phosphatase and human IgG $F(ab')_2$ fragments for phase change immunoassays[122] • Preparing immunogens [123,124]
Sulfo-SMCC Sulfo-succinimidyl 4-(N-maleimidomethyl) cyclo-hexane-1-carboxylate  Sulfo-SMCC	22322	436.37	11.6 Å	Water-soluble analog of SMCC; very stable maleimide-reactive group. This noncleavable cross-linker is membrane impermeable.	• A comparison of maleimide containing heterobifunctional cross-linkers in the conjugation of Fab' fragments to horseradish peroxidase[126] • Preparation of enzyme-antibody conjugates[127]
MBS m-Maleimidobenzoyl-N-hydroxysuccinimide ester  MBS	22310	314.2	9.9 Å	Water-insoluble, noncleavable cross-linker	• Preparing an insulin-ß-galactosidase conjugate[128] • Conjugating hen egg ovalbumin with thiolated synthetic copolymers of D-glutamic acid and D-lysine[129] • Preparing antibody-ß-galactosidase conjugates[130] • Producing ricin immunotoxins[131, 142] • Preparing hapten-carrier protein conjugates from peptides[132, 137, 138, 140, 141] • Coupling blasticidin S to bovine serum albumin[133] • Preparing Fab'-ß-galactosidase conjugates[134] • Preparing synthetic peptide antigens for making antibodies to detect oncogene-related proteins[135] • Investigating the mechanism of cytotoxicity of diphtheria toxin coupled to anti-CD3 MAb[136] • Preparing enzyme labeled viomycin[139]

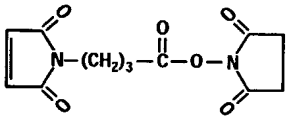
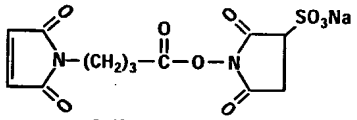
cross-linking

Table 5: NHS-Ester-Maleimide Heterobifunctional Cross-linkers (Continued)

CROSS-LINKER	PRODUCT #	M.W.	SPACER ARM LENGTH	REACTIVITY/ CHARACTERISTICS	APPLICATIONS/ REFERENCES
Sulfo-MBS m-Maleimidobenzoyl-N-hydroxysulfosuccinimide ester  Sulfo-MBS	22312	416.24	9.9 Å	Water-soluble analog of MBS; noncleavable, membrane impermeable.	• An alternative method utilizing small quantities of ligand for affinity purification of monospecific antibodies[143] • Coupling of antibody to β-D-galactosidase[144]
SMPB Succinimidyl 4-(p-maleimido-phenyl)-butyrate  SMPB	22315	356.32	14.5Å	Water-insoluble, extended spacer arm to limit steric hindrance; noncleavable.	• Conjugation of preformed vesicles and Fab' fragments in a study of liposomes as a carrier system[144] • Attaching insulin molecules to reconstituted Sendai virus envelopes[145] • Targeting of loaded Sendai virus envelopes by covalently attached insulin molecules to virus receptor-depleted cells[146] • Forming alkaline phosphatase-Fab' fragment conjugates for an enzyme immunoassay system[147] • Preparing peptide-protein immunogens[148]
Sulfo-SMPB Sulfosuccinimidyl 4-(p-maleimidophenyl)-butyrate  Sulfo-SMPB	22319	458.36	14.5Å	Water-soluble analog of SMPB. Extended spacer arm to limit steric hindrance; non-cleavable, membrane impermeable.	• Studying the transport of the varient surface glycoprotein of Trypanosome brucia[149] • Using aromatic cross-linkers such as Sulfo-SMPB to improve the yield of immunotoxin conjugates[142]
BMH Bismaleimidohexane  BMH	22319	276.29	16.1Å	Water-insoluble homobifunctional cross-linker employing two maleimide functional groups; noncleavable.	• Structural and functional studies of cross-linked Go_{150} protein subunits[150] • Studies of lymphocyte function-associated antigen-3 (LFA-3)[151] • Producing multimeric forms of CD4[152]

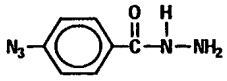
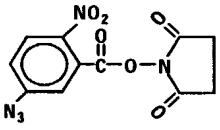
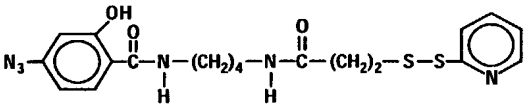
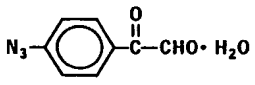
cross-linking

Table 5: NHS-Ester-Maleimide Heterobifunctional Cross-linkers (Continued)

CROSS-LINKER	PRODUCT #	M.W.	SPACER ARM LENGTH	REACTIVITY/ CHARACTERISTICS	APPLICATIONS/ REFERENCES
GMBS N-(g-maleimidobutyryloxy) succinimide ester  GMBS	22314	280.24	10.2 Å	Water-insoluble, noncleavable.	• Acylation of antibody to introduce maleimide groups[153]
Sulfo-GMBS N-(g-maleimidobutyryloxy) sulfosuccinimide ester  Sulfo-GMBS	22324	382.28	10.2 Å	Water-soluble analog of GMBS; noncleavable, membrane impermeable.	154-155

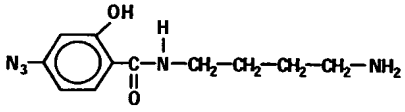
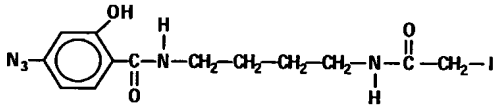
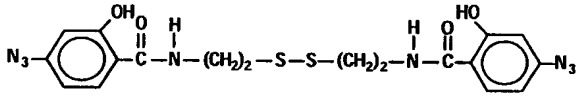
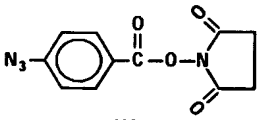
cross-linking

Table 9: Photoreactive Cross-linkers

CROSS-LINKER	PRODUCT #	M.W.	SPACER ARM LENGTH	REACTIVITY	APPLICATIONS/ REFERENCES
ABH Azidobenzoyl Hydrazide  ABH	21510 21509	177.17		• Hydrazide • Phenylazide	• Glycoprotein receptor studies[168]
ANB-NOS N-5-Azido-2-nitrobenzoyloxysuccinimide  ANB-NOS	21551	305.21	7.7 Å	• NHS-ester • Phenylazide	• Cross-linking cobra venom phospholipase A2 aggregation state[169] • Photo-cross-linking of the signal sequence of nascent preprolactin to a polypeptide of the signal recognition particle[170]
APDP N-[4-(p-azidosalicylamido) butyl]-3'(2'-pyridyldithio) propionamide  APDP	27720	446.55		• Pyridyl disulfide • Phenylazide	• Cross-linking of protein subunits and ligand by introduction of disulfide bonds[171]
APG *p*-Azidophenyl glyoxal monohydrate  APG	20107	193.16	9.3 Å	• Phenylazide • Phenyl glyoxal	• Inhibiting bovine heart lactic dehydrogenase, eggwhite lysozyme, horse liver alcohol dehydrogenase, and yeast alcohol dehydro genase[172] • Cross-linking ribonucleic acid-protein in E. coli ribosomes[173] • Identifying regions of brome mosaic virus coat protein chemically cross-linked in situ to viral RNA[174]

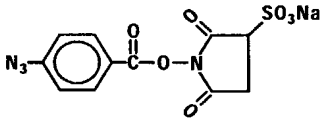
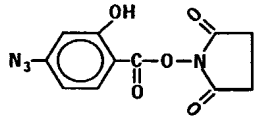
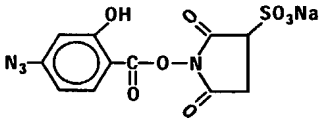
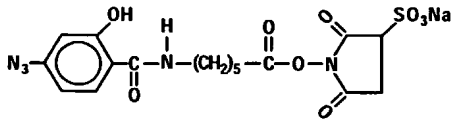
cross-linking

Table 9: Photoreactive Cross-linkers (Continued)

CROSS-LINKER	PRODUCT #	M.W.	SPACER ARM LENGTH	REACTIVITY	APPLICATIONS/ REFERENCES
ASBA 4-(*p*-Azidosalicyl-amido)butylamine  ASBA	21512	249.27	16.3 Å	• Carbonyl reactive • Phenylazide	
ASIB 1-(*p*-Azidosalicyl-amido)-4-(iodo-acetamido)butane  ASIB	21511	417.21	18.8 Å	• Iodoacetyl • Phenylazide	
BASED Bis-[β-4-azidosalicylamido)ethyl] disulfide  BASED	21564	474.54		• Phenylazide (homobifunctional)	• Receptor location • Near neighbor analysis • Protein structural studies • Appropriate in the absence of primary amines and thiols
HSAB N-Hydroxysuccinimidyl-4-azidobenzoate  HSAB	21560	260.21	8.0 Å	• NHS-ester • Phenylazide	• Photoaffinity labeling of peptide hormone binding sites[175] • Photoaffinity labeling of insulin receptor with an insulin analog[176] • Identifying nerve growth factor receptor proteins in sympathetic ganglia membranes[177] • Photoaffinity labeling the hormone receptor of both α and β subunits of human choriogonadotropin[178] • Isolating *in situ* cross-linked ligand-receptor complexes[179] • Cross-linking vasoactive intestinal polypeptide to its receptors on intact human lymphocytes[75]

cross-linking

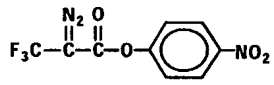
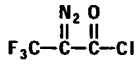
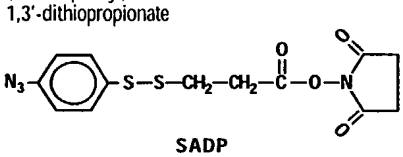
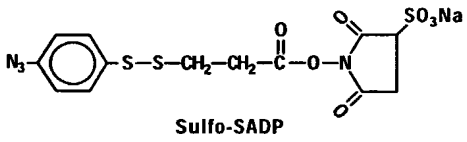
Table 9: Photoreactive Cross-linkers (Continued)

CROSS-LINKER	PRODUCT #	M.W.	SPACER ARM LENGTH	REACTIVITY	APPLICATIONS/ REFERENCES
Sulfo-HSAB N-Hydroxysulfo-succinimidyl 4-azidobenzoate  **Sulfo-HSAB**	21561	362.25	9.0 Å	• NHS-ester • Phenylazide	• Photoaffinity labeling of peptide hormone binding sites[175] • See applications for HSAB
NHS-ASA N-Hydroxysuccinimidyl-4-azidosalicylic acid  **NHS-ASA**	27715	276.21	8.0 Å	• NHS-ester • Phenylazide	• Photoaffinity labeling of ^{125}I-AS-Con A to erythrocyte ghosts[180] • Derivatizing human choriogonadotropin with^{125}I-NHS-ASA and photo-cross-linking the αß dimer[181] • Radiolabeling D-glucose and cross-linking the sugar to the human erythrocyte mono saccharide transporter[182] • Photoaffinity labeling of a bacterial sialidase[183]
Sulfo-NHS-ASA N-Hydroxysulfo-succinimidyl-4-azidosalicylic acid  **Sulfo-NHS-ASA**	27725	378.25	8.0 Å	• NHS-ester • Phenylazide	• See applications/references for NHS-ASA
Sulfo-NHS-LC-ASA Sulfosuccinimidyl-(4-azidosalicylamido)-hexanoate  **Sulfo-NHS-LC-ASA** **M.W. 491.41** **Spacer Arm 18Å**	27735	491.41	18 Å	• NHS-ester • Phenylazide	• See applications/references for NHS-ASA

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cross-linking

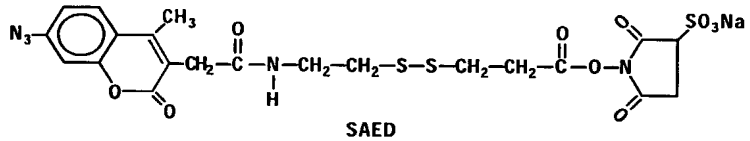
Table 9: Photoreactive Cross-linkers (Continued)

CROSS-LINKER	PRODUCT #	M.W.	SPACER ARM LENGTH	REACTIVITY	APPLICATIONS/ REFERENCES
PNP-DTP *p*-Nitrophenyl- 2-diazo-3,3,3-trifluoropropionate  PNP-DTP	20669	276.15		• Diazo	• Photoaffinity labeling of thyroid hormone nuclear receptors in intact cells[184,185]
DTP 2-Diazo-3,3,3,-trifluoro- propionylchloride  DTP	20670			• Sulfhydryls • Amines	• Pierce offers this product for researchers who require the acid chloride precursor of PNP-DTP
SADP N-succinimidyl- (4-azidophenyl) 1,3'-dithiopropionate  SADP	21552	352.38	13.9 Å	• NHS-ester • Phenylazide	• Cross-linking concanavalin A to receptors on the human erythrocyte membrane[186] • Preparing photoactivatable glycopeptide reagents for site-specific labeling of lectins[187] • Attaching a Sendai virion envelope and a mouse surface membrane polypeptide on newly infected cells[188] • Cross-linking platelet glycoprotein 1b[102]
Sulfo-SADP Sulfosuccin (4-azidophenyldithio) propionate  Sulfo-SADP	21553	454.45	13.9 Å	• NHS-ester • Phenylazide	• See applications/references for SADPimidyl-

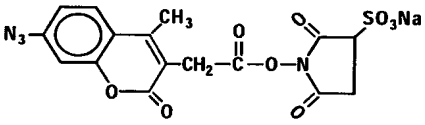
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Table 9: Photoreactive Cross-linkers (Continued)

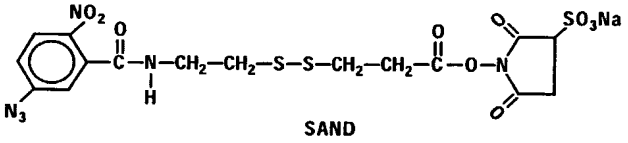
CROSS-LINKER	PRODUCT #	M.W.	SPACER ARM LENGTH	REACTIVITY	APPLICATIONS/ REFERENCES
SAED Sulfosuccinimidyl 2-(7-azido-4-methylcoumarin-3-acetamide) ethyl-1,3'-dithiopropionate	33030	621.60	23.6 Å	• NHS-ester • Phenylazide • Fluorescent	• Functionally directed region specific fluorescent labeling of proteins[189] • Assessing conformational changes in the foot protein of the sarcoplasmic reticulum by site-directed fluorescent labeling[190]
Sulfo-SAMCA Sulfosuccinimidyl 7-azido-4-methylcoumarin-3-acetate	33025	458.34	12.8 Å	• NHS-ester • Phenylazide • Fluorescent	• Specific fluorescent labeling
SAND Sulfosuccinimidyl 2-(m-azido-o-nitrobenzamido)-ethyl-1,3'-dithiopropionate	21549	570.52	18.5 Å	• NHS-ester • Phenylazide	• Demonstration of the aggregation state of Phospholipase A_2[169]



SAED



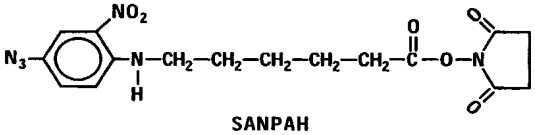
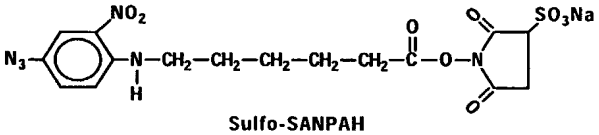
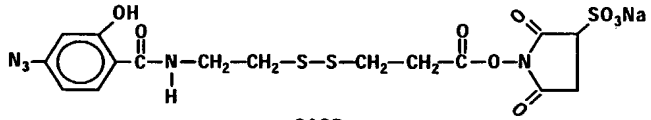
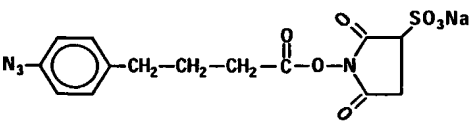
Sulfo-SAMCA



SAND

cross-linking

Table 9: Photoreactive Cross-linkers (Continued)

Cross-Linker	Product #	M.W.	Spacer Arm Length	Reactivity	Applications/References
SANPAH *N*-succinimidyl-6-(4'-azido-2'-nitrophenyl-amino)hexanoate  SANPAH	22588	390.95	18.2 Å	• NHS-ester • Phenylazide	• Cross-linking ligand-receptor complexes *in situ*[179] • Preparing photoactivatable glycopeptide reagents for site-specific labeling of lectins[186] • Photoaffinity labeling of the N-formyl peptide receptor binding site of intact human polymorphonuclear leukocytes[191] • Cross-linking vasoactive intestinal peptide to receptors on intact human lymphoblasts[76]
Sulfo-SANPAH Sulfosuccinimidyl 6-(4'-azido-2'-nitrophenylamino)hexanoate  Sulfo-SANPAH	22589	492.39	18.2 Å	• NHS-ester • Phenylazide	• See applications/references for SANPAH
SASD Sulfosuccinimidyl 2-(*p*-azidosalicyl-amido)ethyl-1,3'-dithiopropionate  SASD	27716	541.51	18.9 Å	• NHS-ester • Phenylazide	• Derivatization of bacterial lipopolysaccharide[192] • Identification of the murine interleukin receptor and N-formyl peptide receptor[193] • Comparison of SASD radiolabeling techniques[194] • Cross-linking of factor V and Va to iodinated peptides[195]
Sulfo-SAPB Sulfosuccinimidyl 4-(*p*-azidophenyl)-butyrate  Sulfo-SAPB	21562	404.32	12.8 Å	• NHS-ester • Phenylazide	

cross-linking

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cross-linking

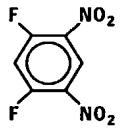
Subunit Cross-linking and Protein Structural Studies

Cross-linkers can be used to study the structure and composition of proteins in biological samples. Some proteins are difficult to study because they exist in different conformations under varying pH or salt conditions. One way to avoid conformational changes is to cross-link the subunits together. Amine-, carboxyl- or sulfhydryl-reactive reagents are employed for identification of particular amino acids or for the determination of the number, location and size of subunits in a protein. Short-to-medium spacer arm cross-linkers are selected when intramolecular cross-linking is performed. If the spacer arm is too long, intermolecular cross-linking can occur. Carbodiimides that result in no spacer arm, along with short length conjugating reagents, such as amine-reactive DFDNB (Product #21524, Table 10) or the photoactivatable amine-reactive cross-linker NHS-ASA (Product #27715), can cross-link between subunits without cross-linking to extraneous molecules if used in optimal concentrations and conditions. Slightly longer cross-linkers such as DMP (Product #20666), DMS (Product #20668), DTBP (Product #20665), DSS (Product #21555) or DSP (Product #22585) can also cross-link between subunits, but they may result in intermolecular coupling. Intermolecular cross-linking can be controlled by adjusting the amount of cross-linker and the concentration of the material to be cross-linked. Dilute protein solutions and high concentrations of cross-linker favor intramolecular cross-linking when homobifunctional cross-linkers are employed. BMH (Product #22319) and other non-cleavable, homobifunctional, sulfhydryl-reactive linkers can be used to link subunits of proteins that were joined by disulfide bonds. After reduction of the disulfides, and by cross-linking through the generated sulfhydryls, the protein will run as its full molecular mass using polyacrylamide gel electrophoresis and reducing conditions. In some circumstances, the cross-linking pattern or success may be affected by the cross-linker's solubility. Hydrophobic cross-linkers tend to cross-link more effectively in hydrophobic regions of molecules.

If the three-dimensional structure of a protein is to be determined or confirmed, cleavable cross-linkers with increasing spacer arm lengths can be used to determine the distance between two subunits. Experiments using cross-linkers with different reactive groups may indicate the locations of specific amino acids. Once conjugated, the proteins are subjected to two-dimensional electrophoresis. In the first dimension, the proteins are run under non-reducing conditions. The molecular weight of the non-reducing sample is recorded. It should be noted that some of the subunits may not be cross-linked and will run according to their individual molecular weights. Other subunits will be combined and, under nonreducing conditions, will run according to the combined molecular weight. The second dimension of the gel is then run using conditions to cleave the cross-linked subunits. The individual molecular weights of the cross-linked subunits can be determined. If the cross-linked subunits were not reduced, the pattern of the second dimension would be a diagonal. However, with the cleavable cross-linker, the cross-linked subunits will be released under reducing conditions, and the individual molecular weights of the subunits will be approximated. The cleaved subunits will be off the diagonal. The molecular weights of the individual subunits should be compared with pre-determined molecular weights of the protein subunits under reducing SDS-polyacrylamide gel electrophoresis.

cross-linking

Table 10: Bifunctional Aryl Halide

CROSS-LINKER	PRODUCT #	M.W.	SPACER ARM LENGTH	REACTIVITY	APPLICATIONS/ REFERENCES
DFDNB 1,5-Difluoro- 2,4-dinitrobenzene  DFDNB	21524	204.1	3 Å	• Aryl halide-amine and sulfhydryl-reactive	• Cross-linking phospholipids in human erythrocyte membranes[196] • Coupling peptides to albumin[197] • Studies of near neighbor relationships of proteins in the myelin membrane[198] • Cross-linking cytochrome oxidase subunits[199]

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cross-linking

Intermolecular Cross-linking for the Study of Protein Interactions and Associations

Cross-linkers are widely used for identification of near-neighbor protein relationships, ligand-receptor identification and interactions, and enzyme-substrate orientations. The cross-linkers chosen for these applications are usually longer than those used for subunit cross-linking. Homobifunctional, amine-reactive NHS-esters or imidates and heterobifunctional, amine-reactive, photoactivatable phenyl azides are the most commonly-used cross-linkers for these procedures. Occasionally, a sulfhydryl- and amine-reactive cross-linker such as Sulfo-SMCC (Product #'s 22522, 22322) may be employed if one of the two proteins or molecules is known to contain sulfhydryls. Cleavable or noncleavable cross-linkers are typically used. Because the distances between two molecules are not always known, the optimum length of the spacer arm of the cross-linker may be determined by the use of a panel of similar cross-linkers with different lengths. DSS (Product #21555) or its cleavable analog DSP (Product #22585) are among the shorter cross-linkers used for protein-protein associations. NHS-ester, phenyl azides are very useful for this type of cross-linking because they usually result in some successful, if not efficient, cross-linking. SASD (Product #27716) is a unique sulfo-NHS-ester, photoactivatable phenylazide that is iodinatable and cleavable. Its characteristics allow for detection and analysis of small quantities of protein.

Cross-linkers can be used to determine whether a particular protein is located on the surface or the integral part of the membrane. These studies are possible because water-soluble cross-linkers are membrane-impermeable, while water-insoluble cross-linkers are membrane-permeable. The experiment can be carried out by performing a conjugation reaction of a particular cell membrane preparation to a known protein or radioactive label in the presence of water-soluble or water-insoluble cross-linkers. Upon conjugation the cells may be washed, solubilized and characterized by SDS-PAGE. The gel electrophoresis results can be used to determine whether the protein of interest was conjugated. Any integral membrane protein will conjugate in the presence of a water-insoluble cross-linker, but not in the presence of water-soluble cross-linkers. Surface membrane proteins should conjugate in the presence of both water-soluble and water-insoluble cross-linkers.

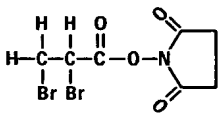
BASED (Product #21564), a homobifunctional photoactivatable phenyl azide, is one of the more versatile cross-linkers for the study of protein interactions and associations. It is cleavable and can be radiolabeled with ^{125}I using IODO-BEADS® Iodination Reagent (Product #28665). After cleavage, both of the dissociated molecules will still be iodinated. Because both reactive groups on this cross-linker are nonspecific, the cross-linking is not dependent on amino acid composition for successful conjugation.

SDBP (Product #22340) is a cross-linker that is amine-reactive at both ends, but contains two different reactive groups with varying reactivity. Please see Table 11 for more information on SDBP. The reaction is controlled by temperature. SDBP is an NHS-ester with amine reactivity that is only slightly affected by temperature; however, its second amine-reactive functional group is a dibromoacetyl group that is slow to react with amines at physiological pH at 4°C. This cross-linker can be useful for studying conformational changes in proteins.

cross-linking

Table 11: Heterobifunctional Amine-Reactive Cross-linker

CROSS-LINKER	PRODUCT #	M.W.	SPACER ARM LENGTH	REACTIVITY	APPLICATIONS/ REFERENCES
SDBP N-Hydroxysuccin-imidyl 2,3-Dibromopropionate	22340	328.96	5.0 Å	• NHS-ester • Alkyl dibromide	• Preparation of immunotoxins[200]



SDBP
M.W. 328.96
Spacer Arm 5.0 Å

References

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cross-linking

Cell Membrane Structural Studies

Cell membrane structural studies require reagents of varying hydrophobicity to determine the location and the environment within a cell's lipid bilayer. Fluorescent tags are used to locate proteins, lipids or other molecules inside and outside the membrane. Various cross-linkers with differing spacer arm lengths can be used to cross-link proteins to associated molecules within the membrane to determine the distance between molecules. Successful cross-linking with shorter cross-linkers is a strong indication that two molecules are interacting in some manner. Failure to obtain cross-linking with a panel of shorter cross-linkers, while obtaining conjugation with the use of longer reagents, generally indicates that the molecules are located in the same part of the membrane but are not interacting. Homobifunctional NHS-esters, imidates or heterobifunctional NHS-ester, photoactivatable, phenyl azides are commonly used for these procedures. Because they are membrane impermeant, sulfo-NHS-esters are not useful for cross-linking within the membrane. Imidoester cross-linkers (imidates) are water-soluble, but they are still able to penetrate membranes. DTBP (Product #20665) is an amine-reactive imidoester that is cleavable by sulfhydryls. Sulfhydryl-reactive cross-linkers may be useful for targeting molecules with cysteines to other molecules within the membrane.

EDC (Product #'s 22980, 22981), water insoluble dicyclohexylcarbodiimide, or DCC (Product #20320), and other water-soluble and water-insoluble coupling reagents are used to study membranes and cellular structure,[52,53] protein subunit structure and arrangement,[54,55] enzyme-substrate interactions,[56-58] and cell surface[59] and membrane receptors.[60,61] The hydrophilic character of EDC can result in much different cross-linking patterns in membrane and subunit studies than with hydrophobic carbodiimides such as DCC.[53,55] Often it is best to attempt cross-linking with a water-soluble and water-insoluble carbodiimide to obtain a complete picture of the spacial arrangements or protein-protein interactions involved.

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cross-linking

Immunotoxins

Specific antibodies can be covalently linked to toxic molecules and then used to target antigens on cells. Often these antibodies are specific for tumor associated antigens. Immunotoxins are brought into the cell by surface antigens and, once internalized, they proceed to kill the cell by ribosome inactivation or other means. The type of cross-linker used to make an immunotoxin can affect its ability to locate and kill the appropriate cells. For immunotoxins to be effective, the conjugate must be stable in vivo. In addition, once the immunotoxin reaches its target, it is important that the antibody be separable from the toxin to allow the toxin to kill the cell. Thiol-cleavable, disulfide-containing conjugates have been shown to be more cytotoxic to tumor cells than noncleavable conjugates of ricin A immunotoxins. Cells are able to break the disulfide bond in the cross-linker, allowing the release of the toxin within the targeted cell.

SPDP (Product #'s 21757, 21657, 21557) is a reversible NHS-ester, pyridyl disulfide cross-linker used to conjugate amine-containing molecules to sulfhydryls. For several years, this has been the "workhorse" cross-linker for production of immunotoxins. The amine-reactive NHS-ester is usually reacted first with the antibody. In general, toxins do not contain surface sulfhydryls; therefore, sulfhydryls must be introduced onto them by reduction of disulfides, which is common for procedures involving ricin A chain and abrin A chain, or through chemical modification reagents. A second SPDP molecule can be used for this purpose. It is reacted with amines on the immunotoxin, then reduced to yield sulfhydryls. Another chemical modification reagent that is commonly used for production of immunotoxins is 2-iminothiolane, also known as Traut's Reagent (Product #26101). Traut's Reagent reacts with amines and yields a sulfhydryl when its ring structure opens during the reaction.

Other water-soluble SPDP analogs, such as Sulfo-LC-SPDP (Product #'s 21650, 21649), are available for immunotoxin production, allowing for ease of use or avoidance of organic solvents. In addition, Sulfo-LC-SPDP and LC-SPDP (Product #'s 21651, 21652) have longer spacer arms and can offer better conjugation efficiency.

SMPT (Product #21558) is a reversible, NHS-ester, pyridyl disulfide cross-linker developed to provide increased stability of immunotoxins *in vivo*. The disulfide bond in SMPT is protected, making it less likely to be cleaved *in vivo* prior to reaching the antigenic target. In addition, the NHS-ester of SMPT is much more stable in aqueous solution than typical NHS-ester compounds, showing little degradation even after several hours in aqueous solution. A water-soluble long chain version of SMPT is also offered—Sulfo-LC-SMPT (Product #'s 21569, 21568).

cross-linking

Carrier Protein Hapten/ Peptide/Polypeptide Conjugates for Use as Immunogens

Pierce offers many products in this area of immunological research. Easy-to-use kits are available for coupling ligands using several different chemistries. These kits and the use of immunogens are discussed in the Antibody Production Technical Section of this catalog. There are many cross-linkers used for the production of these conjugates, and the best choice is dependent on the reactive groups present on the hapten and the ability of the hapten-carrier conjugate to function successfully as an immunogen after its injection. Carbodiimides are good choices for producing peptide carrier conjugates because both proteins and peptides usually contain several carboxyls and primary amines. Carbodiimides such as EDC (Product #'s 22980, 22981) react with carboxyls first to yield highly reactive unstable intermediates. The intermediates can then couple to primary amines. Many different carboxyl- or amine-containing small molecules can be attached to carrier proteins using this easy-to-use chemistry.

Other heterobifunctional cross-linkers can also be used to make immunogen conjugates. Often peptides are synthesized with terminal cysteines to allow for their attachment to supports or to carrier proteins through a part of the molecule that is not important for activity or recognition. Sulfhydryl-reactive, heterobifunctional cross-linkers can be coupled to carrier proteins through their other functional group and then can be linked to peptides through terminal cysteines. This method can be very efficient and yield an immunogen that is capable of eliciting a good response upon injection. A good choice of cross-linker with these characteristics is Pierce's Sulfo-SMCC (Product #22322). This cross-linker is an amine-reactive NHS-ester that contains a cyclohexyl group in its spacer and a very stable maleimide group at the other end of the molecule. The maleimide of Sulfo-SMCC is more stable than the maleimide on other NHS-ester maleimide cross-linkers because of the stability imparted by the cyclohexyl ring. Pierce uses Sulfo-SMCC to produce its entire selection of Maleimide Activated Carrier Proteins and Kits. Please see the Antibody Production Technical Section for additional information. Other cross-linkers that can be used to make immunogens are MBS (Product #'s 22510, 22310), SMPB (Product #'s 22316, 22315) and GMBS (Product #22314). Water-soluble analogs are also available, including Sulfo-MBS (Product #'s 22313, 22312), Sulfo-SMPB (Product #'s 22318, 22317) and Sulfo-GMBS (Product #22324).

SDBP (Product #22340) is a cross-linker that is amine-reactive at both ends, but contains two different reactive groups with varying reactivity. The reaction is controlled by temperature. SDBP is an NHS-ester with amine reactivity that is only slightly affected by temperature; however, its second amine-reactive functional group is a dibromoacetyl group, which is slow to react with amines at physiological pH at 4°C. A possible application for this cross-linker is to allow the NHS group to react with amines on the carrier protein. After quick removal of the excess cross-linker from the carrier protein, an amine-containing hapten can be added to the solution, and the reaction can be allowed to warm to room temperature, then proceed for several hours.

cross-linking

Solid-Phase Immobilization

Proteins, peptides and other molecules can be immobilized on solid-phase matrices for use as affinity supports or for sample analysis. The matrices may be agarose, beaded polymers, polystyrene plates or balls, porous glass or glass slides, and nitrocellulose or other membrane materials. Some supports can be activated for direct coupling to a ligand. Other supports are made with nucleophiles or other functional groups that can be linked to proteins or other ligands using cross-linkers. Carbodiimides such as DCC (Product #20320) and EDC (Product #'s 22980, 22981) are very useful for coupling proteins to carboxy- and amine-activated glass, plastic and agarose supports. Carbodiimide procedures are usually one-step methods; however, two-step methods are possible if reactions are performed in organic solvents, or if NHS (Product #24500) or Sulfo-NHS (Product #24510) are used to enhance the reaction.

EDC is useful for coupling ligands to solid supports.[62-66] It can also be used to attach leashes onto affinity supports and for subsequent coupling of ligands. Useful spacers are diaminodipropylamine (DADPA),[62] ethylenediamine, hexanediamine,[63,64] 6-amino-caproic acid,[62,65] and any of several amino acids or peptides.[62] Useful solid supports for immobilization are agarose,[62-65] plastic,[62] or cellulose matrices.[66] Leashes become necessary to overcome steric effects when the ligand is immobilized too near the matrix to allow access by the molecule to be bound. Steric effects are usually most pronounced when the ligand is a small molecule. Reaction times are generally in the range of 1-3 hours for EDC coupling of molecules to solid supports. The amide bond formed by EDC coupling is relatively stable, especially at neutral pH.

Heterobifunctional cross-linkers that can be reacted in two-steps are often more useful and efficient for producing solid-phase supports than homobifunctional cross-linkers. Amine-activated supports can be converted to sulfhydryl-reactive supports using NHS-ester maleimide cross-linkers such as Sulfo-SMCC (Product #'s 22522, 22322). For some compounds that are difficult to immobilize, it may be possible to use NHS-ester, photoactivatable, phenyl azides to attach them to amine-activated supports. The photoactivatable, phenyl azide is unreactive in the dark but, once exposed to the appropriate wavelength range of light, it becomes extremely reactive and able to nonselectively couple to almost any ligand.

The cross-linker DMP (Product #20666) has been employed in the production of immobilized antibodies on protein A or protein G columns for use as antigen purification supports.[39] After antibody binds to the Fc-binding proteins, most or all of the antibody can be oriented so that the Fab region is available for antigen recognition. DMP is applied to the bound antibody column to link the two proteins through primary amines.

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cross-linking

Protein-Protein Conjugates

One of the most widely used applications for cross-linkers is the production of protein-protein conjugates. Biological assays require methods for detection, and one of the most common methods for quantitation of results is to conjugate an enzyme, fluorophore or other molecule to a protein that has affinity for one of the components in the biological system being studied. Antibody-enzyme conjugates (primary or secondary antibodies) are among the most common protein-protein conjugates used. Secondary antibodies are relatively inexpensive and are available from Pierce (see the Antibody Ordering Section of this catalog). However, enzyme labeled primary antibodies are usually expensive and can be difficult to obtain. Many researchers find it necessary to label their primary antibodies.

There are many reagents used for the production of antibody-enzyme conjugates. These have been produced by glutaraldehyde cross-linking in one- and two-step procedures. These conjugates are easy to make but often yield conjugates that give high background in immunoassays. Carbohydrate moieties of antibodies can be oxidized and then coupled to primary amines on enzymes, such as horseradish peroxidase, in a procedure called reductive alkylation or amination. These conjugates give less background in enzyme immunoassays and are relatively easy to prepare. Some self-conjugation of antibody may occur in the protocol. Homobifunctional NHS-ester or imidoester cross-linkers can be substituted for glutaraldehyde in a one-step protocol; however, polymerization and self-conjugation are still likely to occur. Homobifunctional sulfhydryl-reactive cross-linkers such as BMH (Product #22319) and DPDPB (Product #21701) may be useful if both proteins to be conjugated contain sulfhydryls.

Heterobifunctional cross-linkers are perhaps the best choices for antibody-enzyme or other protein-to-protein cross-linking. Unwanted self-conjugation inherent when using homobifunctional NHS-ester reagents or glutaraldehyde can be avoided when using a reagent such as SMCC (Product #'s 22321, 22320) or Sulfo-SMCC (Product #'s 22522, 22322). Sulfo-SMCC is conjugated to one protein, and the second is thiolated with SATA (Product #26102) or Traut's Reagent (Product #26101). Alternatively, disulfides in the protein are reduced, and the two activated proteins are incubated together to form conjugates that are free of dimers of either protein. Any of the other NHS-ester maleimide or pyridyl disulfide cross-linkers can be substituted for Sulfo-SMCC in this reaction scheme. Heterobifunctional photoactivatable phenylazide cross-linkers are seldom used for making protein-protein conjugates because conjugation efficiencies

cross-linking

DNA/RNA Cross-linking to Proteins

Cross-linking of DNA or RNA to proteins is more limited because the reactivities of most cross-linkers favor protein-protein cross-linking over protein-DNA cross-linking. To assist in these cross-linking methods, DNA probes are often synthesized with primary amines or thiols attached to specific bases. After insertion of the bases into DNA, amine- or sulfhydryl-reactive cross-linkers can be used for their conjugation to proteins. EDC (Product #'s 22980, 22981) has been reportedly used to cross-link RNA to ribosomal protein subunits. Other specialized chemistries are reviewed in Wong's book, *Chemistry of Protein Conjugation and Cross-linking* (Product #15010).

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cross-linking

Other Applications

There are many additional applications for cross-linkers that are either antiquated methods, new technologies or for more specialized needs. Older methods for peptide synthesis involve use of carbodiimide cross-linkers such as DCC (Product #20320) and EDC (Product #'s 22980, 22981) for the step-wise addition of individual amino acids to support bound peptides. Cross-linkers such as glutaraldehyde and dimethylpimelimidate have been used for tissue fixation. Newer cross-linkers are being developed that have more than two functional groups. Some trifunctionals are already reported in the literature.

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- APPENDIX B –

TOTAL SYNTHESIS OF *N*-LINKED GLYCOPOLYPEPTIDES:

DESIGN AND PREPARATION OF ARTIFICIAL HIV ANTIGENS BASED ON THE 2G12 EPITOPE

COMPLICATING FACTORS IN CREATING EFFICIENT HIV VACCINE

- Low antigenicity of the envelope spike surface (very little of surface is available for antibody binding)
 - high degree of glycosylation
 - oligomerization of constituent proteins
- Low immunogenicity of the envelope proteins (the mature oligomer typically stimulates only weak antibody response)
 - most of the epitopes on unprocessed gp160 are unaccessible on mature envelope
 - strong initial response to gp160 may supress response to lower concentrations of mature oligomer
 - "glycan shield" problem
 - conformational masking of receptor binding sites
- High degree of viral variation

Burton *Proc. Natl. Acad. Sci. USA* **1997**(94) 10018-10023
Kwong et al. *Nature* **2002**(420) 678-682
Wei et al. Nature **2003**(422) 307-312

MAJOR STRATEGIES FOR CANDIDATE VACCINE DESIGN

1. Vaccination with attenuated virus

 + *typically mature oligomer is displayed*

 − *safety issues*
 − *low immunogenicity of viral oligomer*

2. Vaccination with virus in an inactivated form

 + *mature oligomer may be displayed*
 + *less safety concerns*

 − *inactivation difficult without conformation disruption*
 − *low immunogenicity*

3. DNA immunization

 + *mature oligomer may be displayed*

 − *needs a suitable carrier*
 − *needs to ensure efficient gp160 processing*

4. Immunization with a recombinant oligomeric molecule

 + *immunization with mature oligomer*

 − *hard to fully reproduce correct oligomer conformation*
 − *low immunogenicity*

5. Preparation and immunization with epitope mimics of potent neutralizing antibodies

 + *highly focused response could potentially be elicited*

 − *difficult to produce appropriate epitope mimics (especially for discontinuous epitopes)*

ADVANTAGES OF TARGETING ENVELOPE GLYCANS

The target carbohydrate epitope is

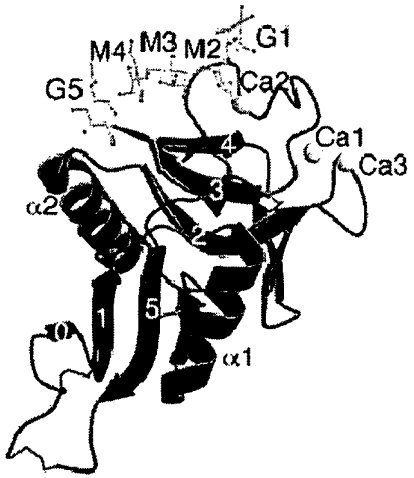
- ✓ located on the "silent face" of gp120
- ✓ easily accessible on mature trimeric gp41-gp120 complex
- ✓ does not overlap with receptor binding sites
- ✓ "entropically disfavored binding" problem is minimized

onserved carbohydrate epitopes can be utilized

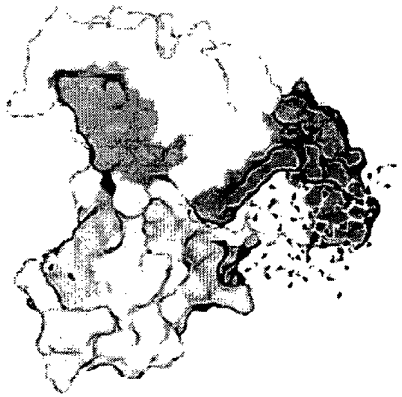
bility to use HIV "Glycan Shield" defense mechanism against the virus

MOLECULES BINDING GP120 CARBOHYDRATES

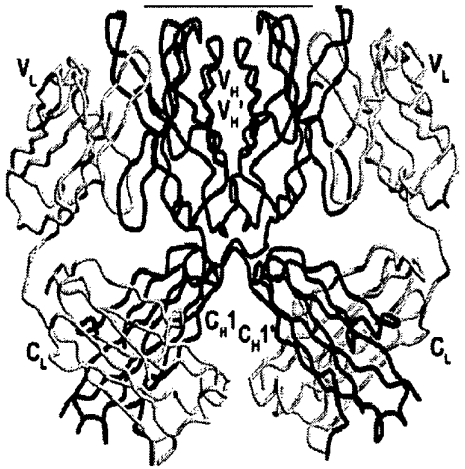
DC-SIGN

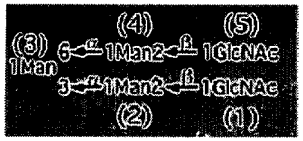


Cyanovirin-N



2G12 Fab



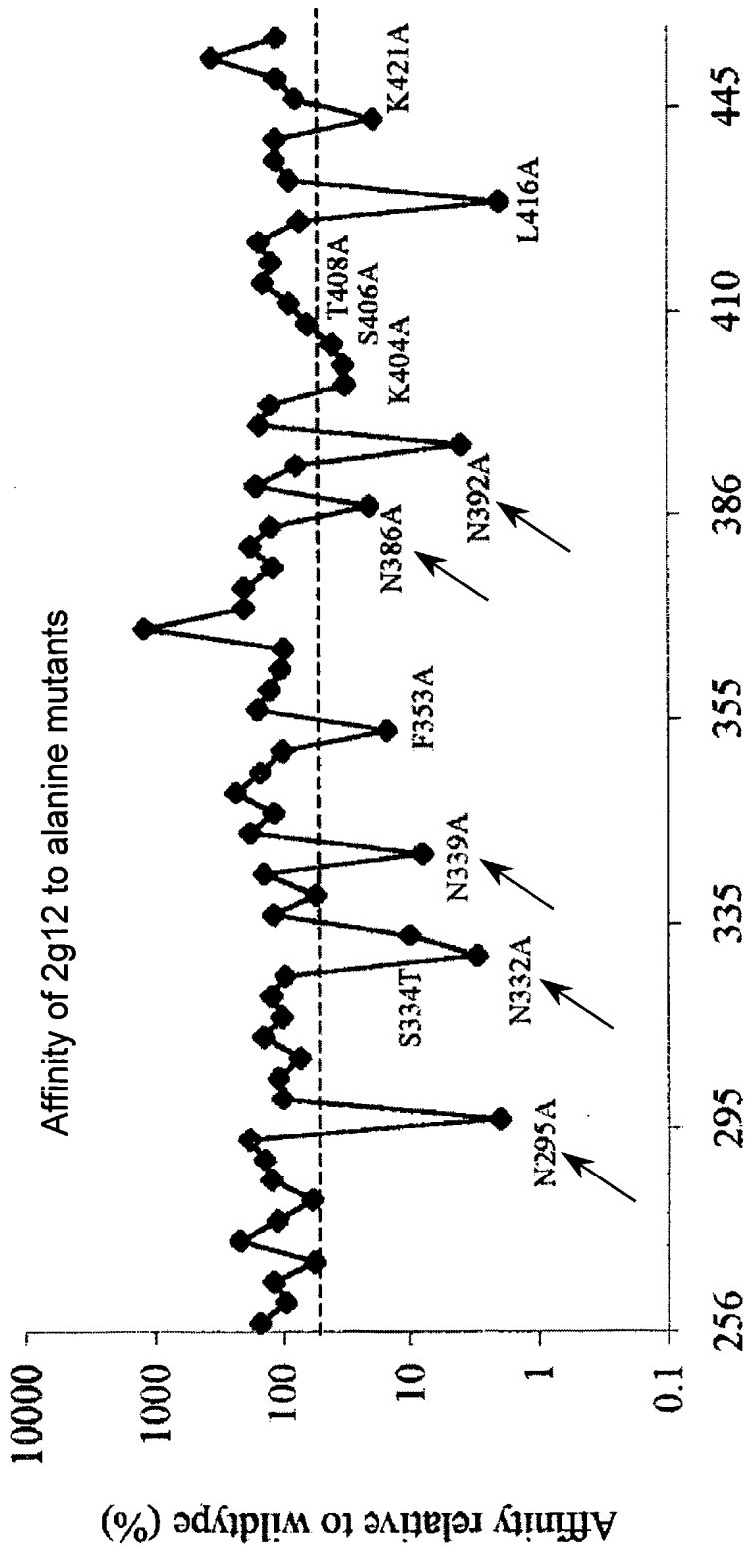


high-mannose

high-mannose/
hybrid

Feinberg et al. *Science* **2001** (294) 2163-2166
Botos et al. *J. Biol. Chem* **2002** (227) 34336-34342
Sanders et al. *J. Virology* **2002** (76) 7293-7305
Scanlan et al. *J. Virology* **2002** (76) 7306-7321

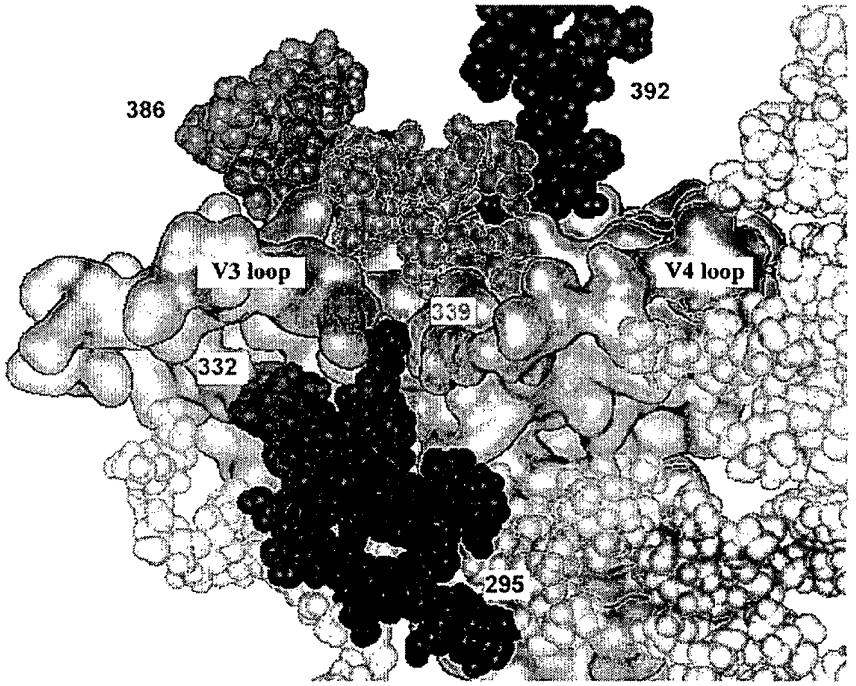
MAPPING OF THE 2G12 EPITOPE ON GP120: AA SEQUENCE



Scanlan et al. *J. Virology* **2002** (76) 7306-7321

MAPPING OF THE 2G12 EPITOPE ON GP120: AA SEQUENCE

Mutation[a]	Affinity of antibody for indicated mutant relative to wild type (%)	
	2G12	IgG1 b12
N295A	2	80
N295Q	4	261
N332A	3	75
N339A	8	30
N339Q	91	567
N386A	21	23
N392A	4	4
N392Q	6	142
S334T	10	6
[illegible]6A	2	2
[illegible]3A	15	29
[illegible]1A	19	18
[illegible]4A	32	101
[illegible]6A	33	60
[illegible]8A	40	96
[illegible]5A	1,173	667
[illegible]8A	349	857



Summary:

- Binding is sensitive to mutations at N295, N332, N339, N386, N392
- **N295, N332, N392 are highly conserved across a broad range of primary isolates**
- 1-2 α-mannose linkages are important for 2g12 binding

Conclusion:

➢ Epitope is formed from high mannose type glycans at N295 and N332

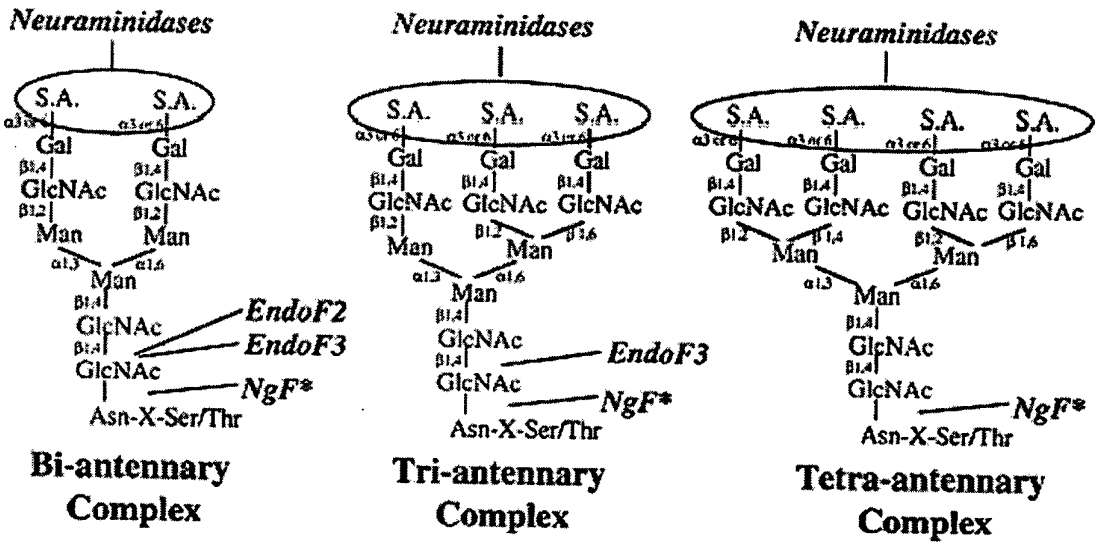
Scanlan et al. *J. Virology* **2002** (76) 7306-7321

MAPPING OF THE 2G12 EPITOPE ON GP120: GLYCAN STRUCTURE

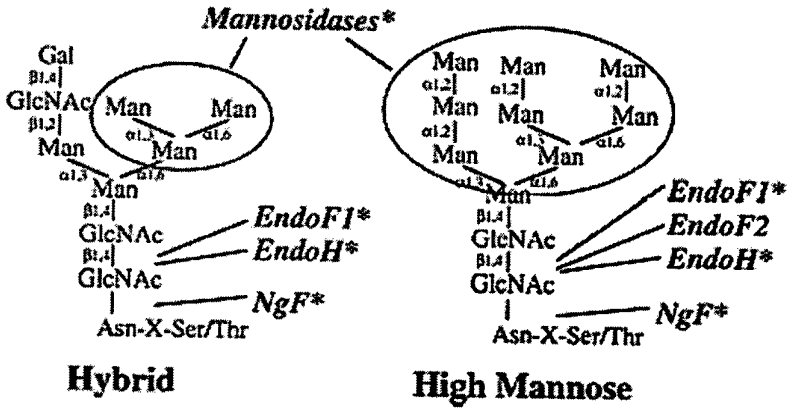
2G12 Binding to deglycosylated gp120:

NgF
Endo F1
Endo H
α-mannosidase

:te disruption of 2G12 binding;
ect on b12



Endo F2: no effect

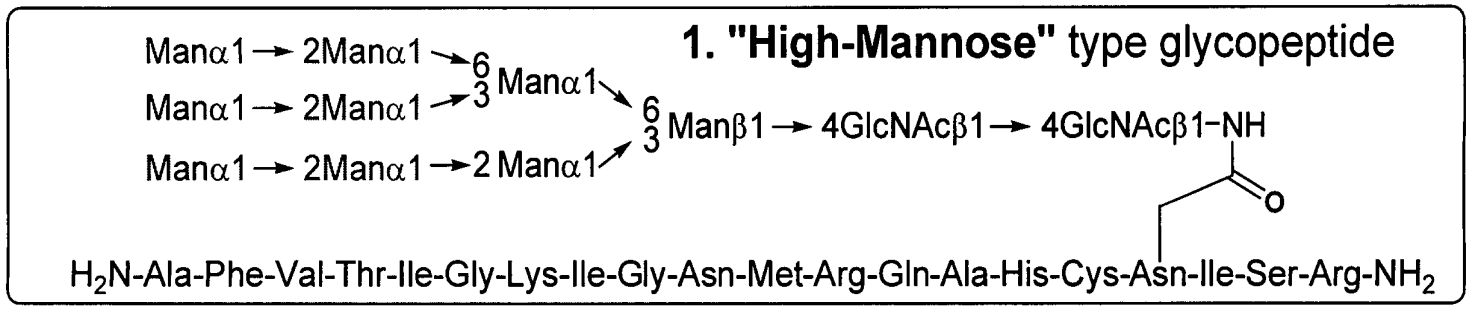


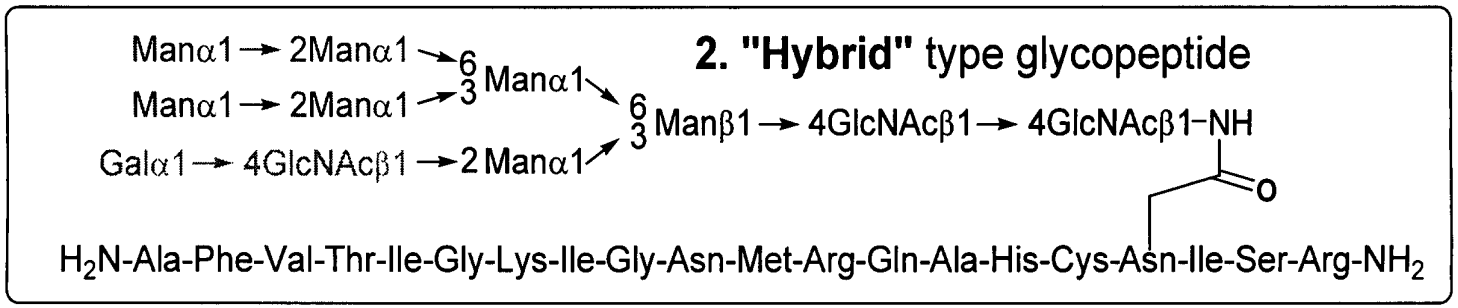
Conclusion:
Glycans involved in the epitope are more likely to be ***hybrid*** than ***high-mannose*** type

Sanders et al., *J Virology* **76**(14) 2002 p. 7293

FIRST GENERATION TARGET GLYCOPEPTIDES

HIV gp120$^{316-335}$ Glycopeptide Fragments





ADVANTAGES OF THE TOTAL SYNTHESIS STRATEGY

Total synthesis vs. Isolation form natural sources

1. Homogeneous, defined glycans are produced
 - *the molecule is made step by step, the structure is controlled throughout the synthesis*
 - *microheterogeneiety problem is eliminated*
 - *better defined therapeutic formulations*

2. Larger quantities available
 - *hundreds of milligrams can be obtained compared with submilligram quantities typical for glycoprotein purification*

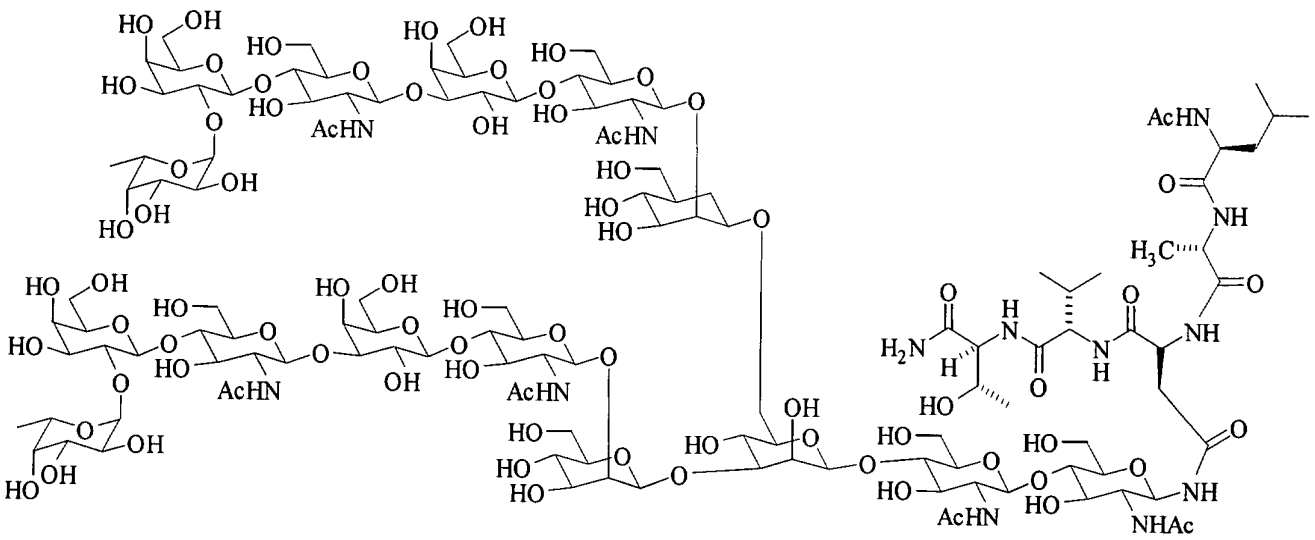
Lower cost

.. Structural modifications can easily be introduced

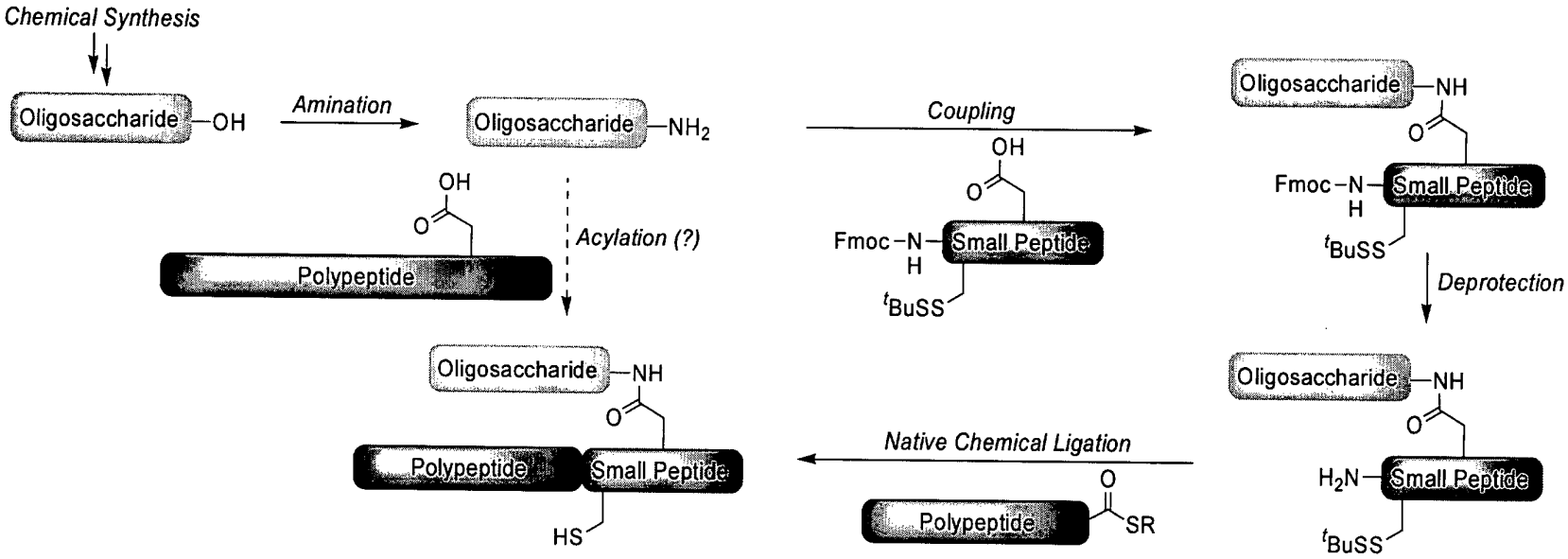
5. Glycomimetics and unnatural glycans can only be prepared by synthesis, and possibly used to
 - *improve stability*
 - *overcome the "degeneracy in carbohydrate recognition" problem*
 - *help induce cellular immune response*

"N-LINKED" PROJECT IN DANISHEFSKY LABORATORIES

- Glycal Assembly technology
- Solid phase oligosaccharide synthesis
- Synthesis of "Symmetrically" branched complex type glycans
- Preparation 15-mer glycan carrying H-type blood group determinants:

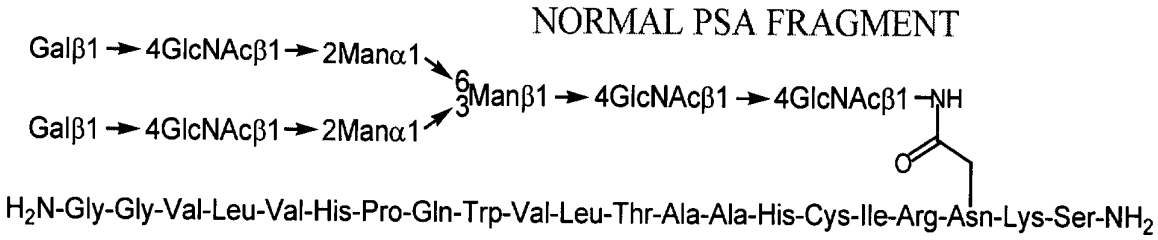


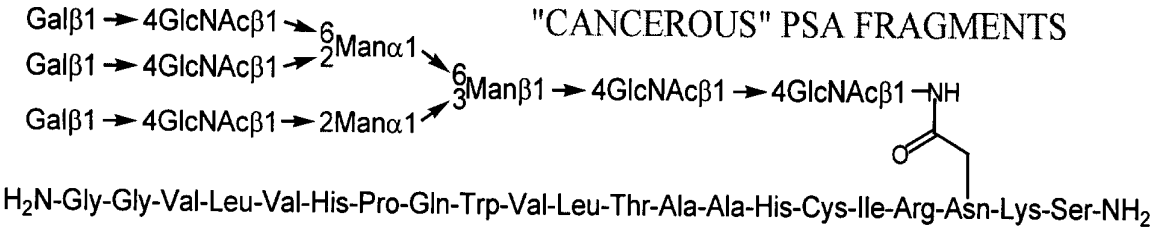
GENERAL APPROACH TO N-LINKED GLYCOPEPTIDES

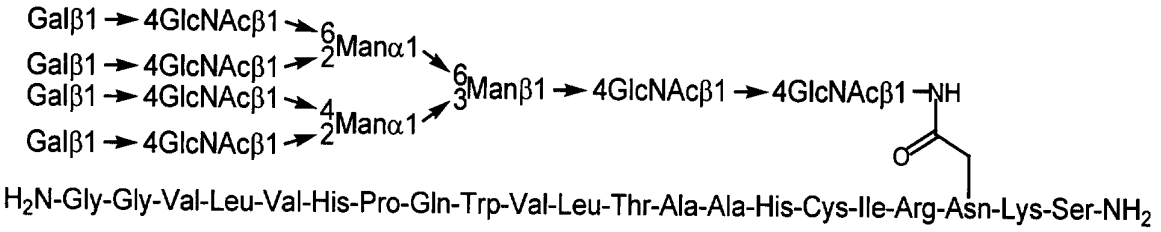


Miller J., Dudkin V., Lyon G., Muir T., and Danishefsky J. Toward fully synthetic *N*-linked glycoproteins. *Angew. Chem. Int. Ed.* 2003; 42(4), 431–434

normal and transformed PSA$^{27-47}$ glycoforms



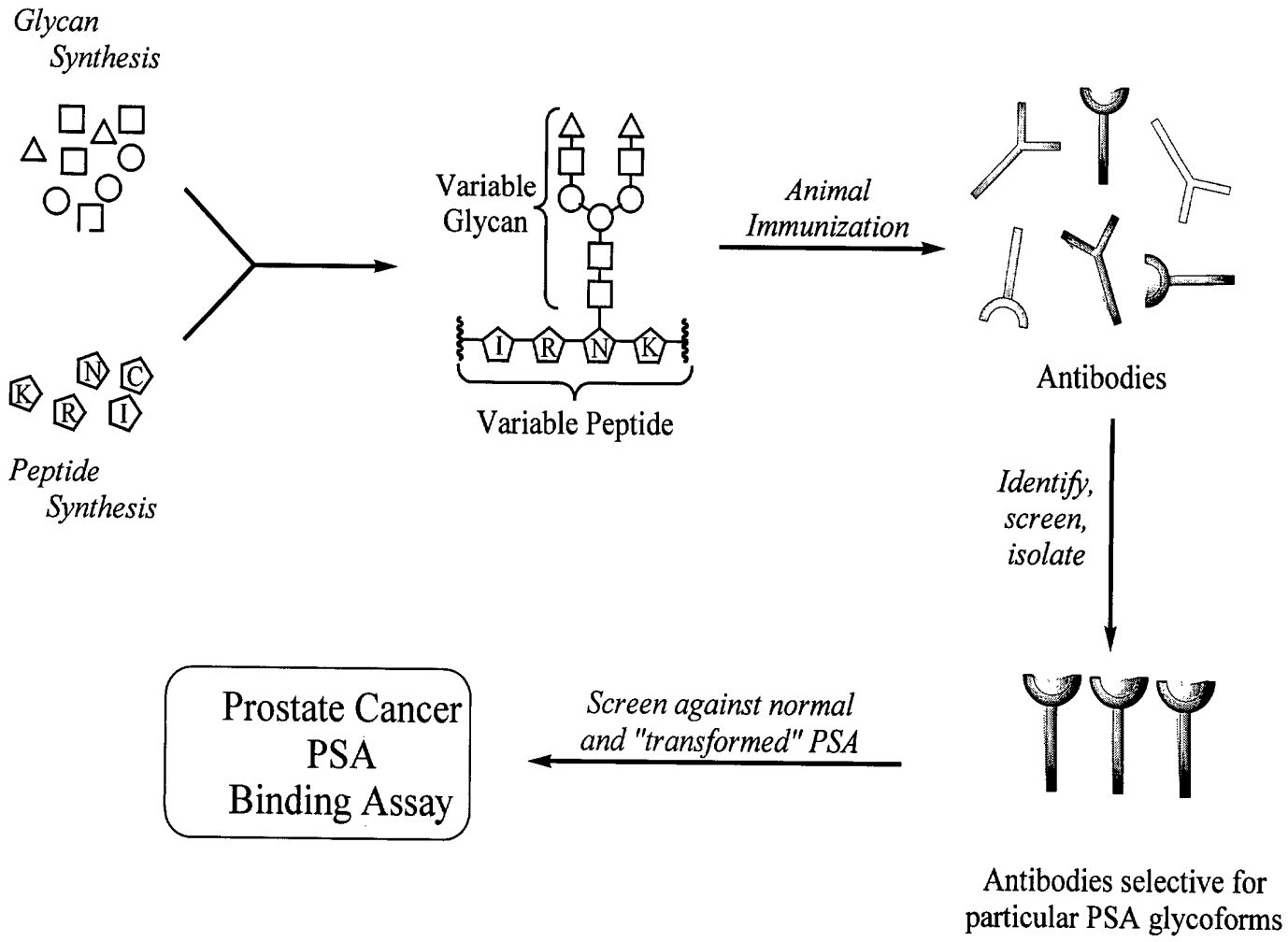




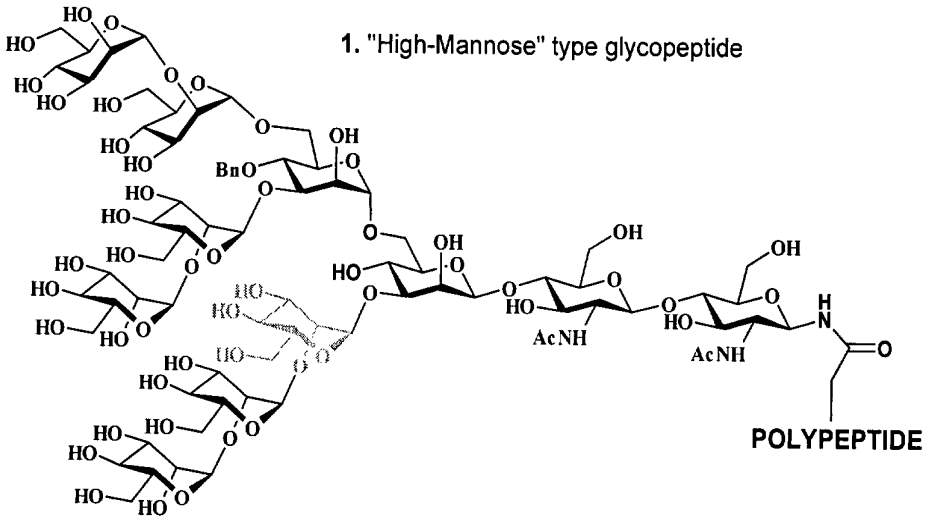
Okada et al *Biochim. Biophys. Acta-Gen. Subj.* **2001**, 1525, 149-160
Prakash, S.; Robbins, P. W. *Glycobiology* **2000**, *10*, 173-176.

WO 2004/050711
PCT/US2003/038471

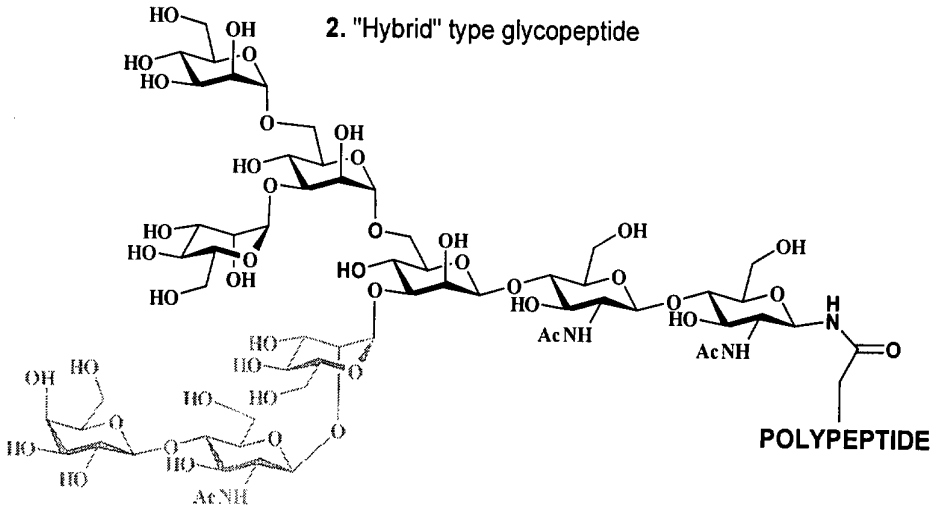
A POTENTIAL PROSTATE CANCER DIAGNOSTIC ASSAY



CORE SUBSTITUTION IN GP120 AND PSA CARBOHYDRATES



2, 3', 6'



2, 3', 6'

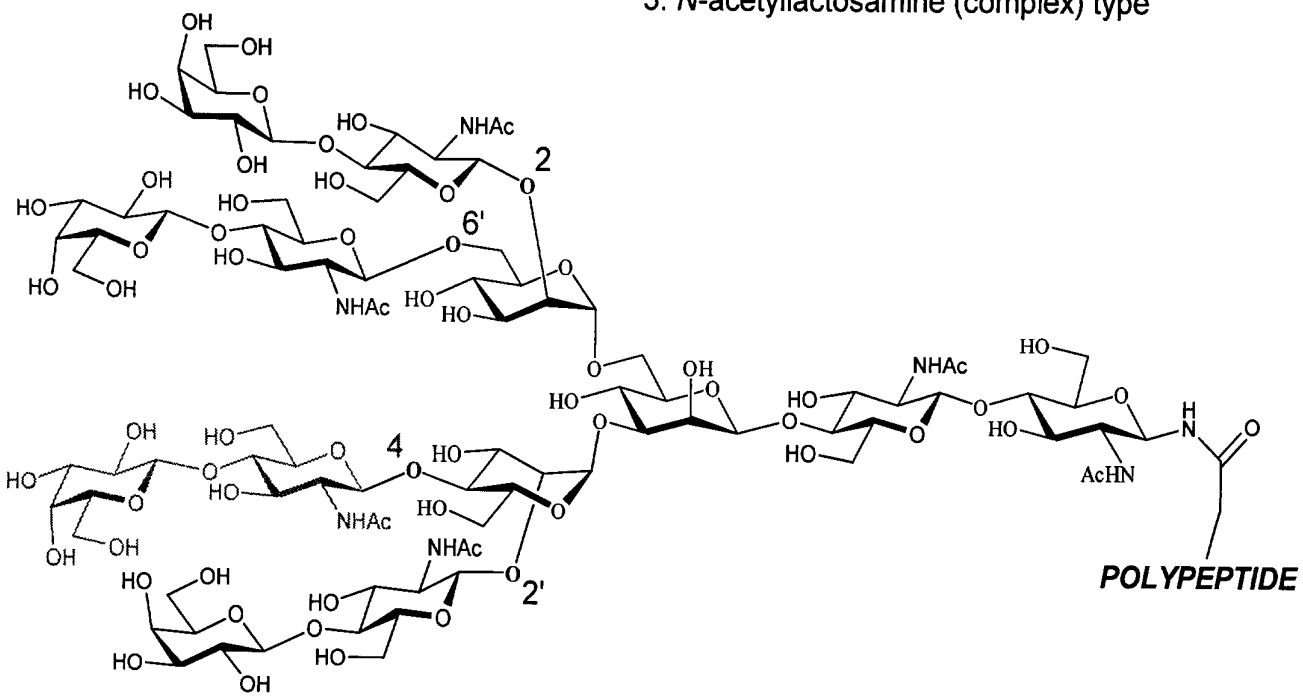
CORE SUBSTITUTION IN GP120 AND PSA CARBOHYDRATES

3. *N*-acetyllactosamine (complex) type

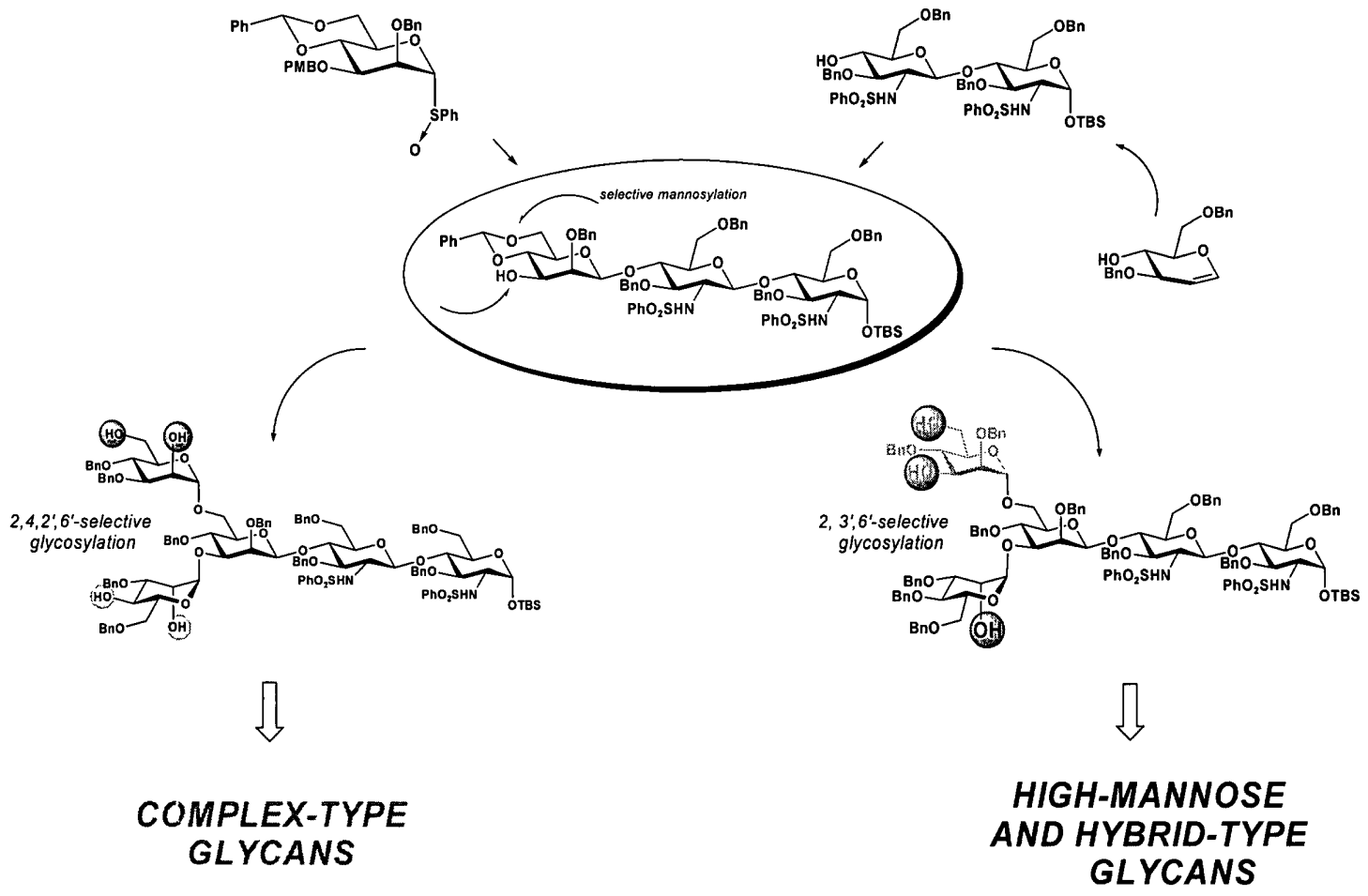
2, 2'

2, 4, 2'

2, 4, 2', 6'

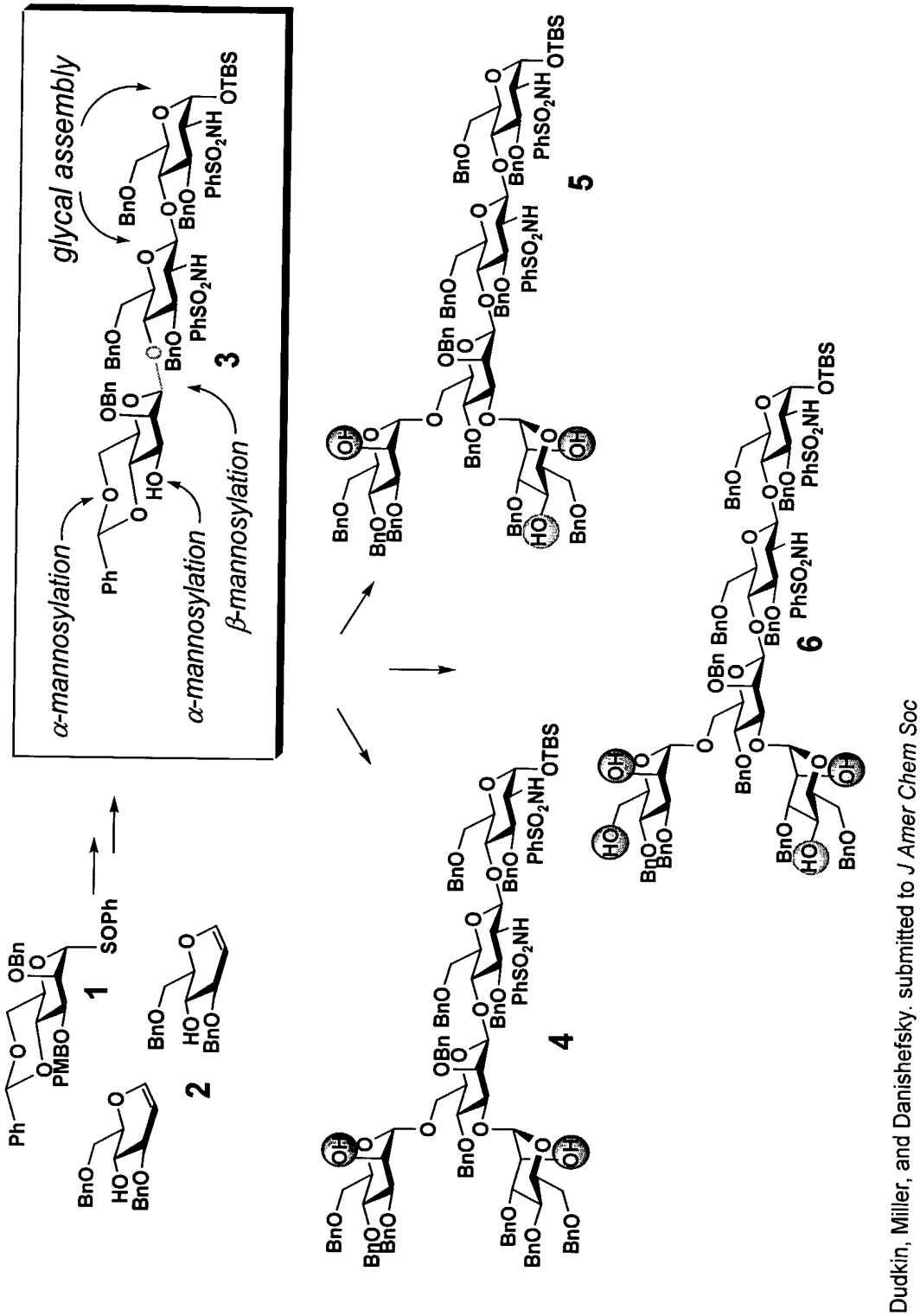


NOVEL SYNTHETIC STRATEGY: BUILDING ASYMMETRICALLY BRANCHED GLYCANS



SYNTHESIS OF PSA$^{27-47}$ FRAGMENTS

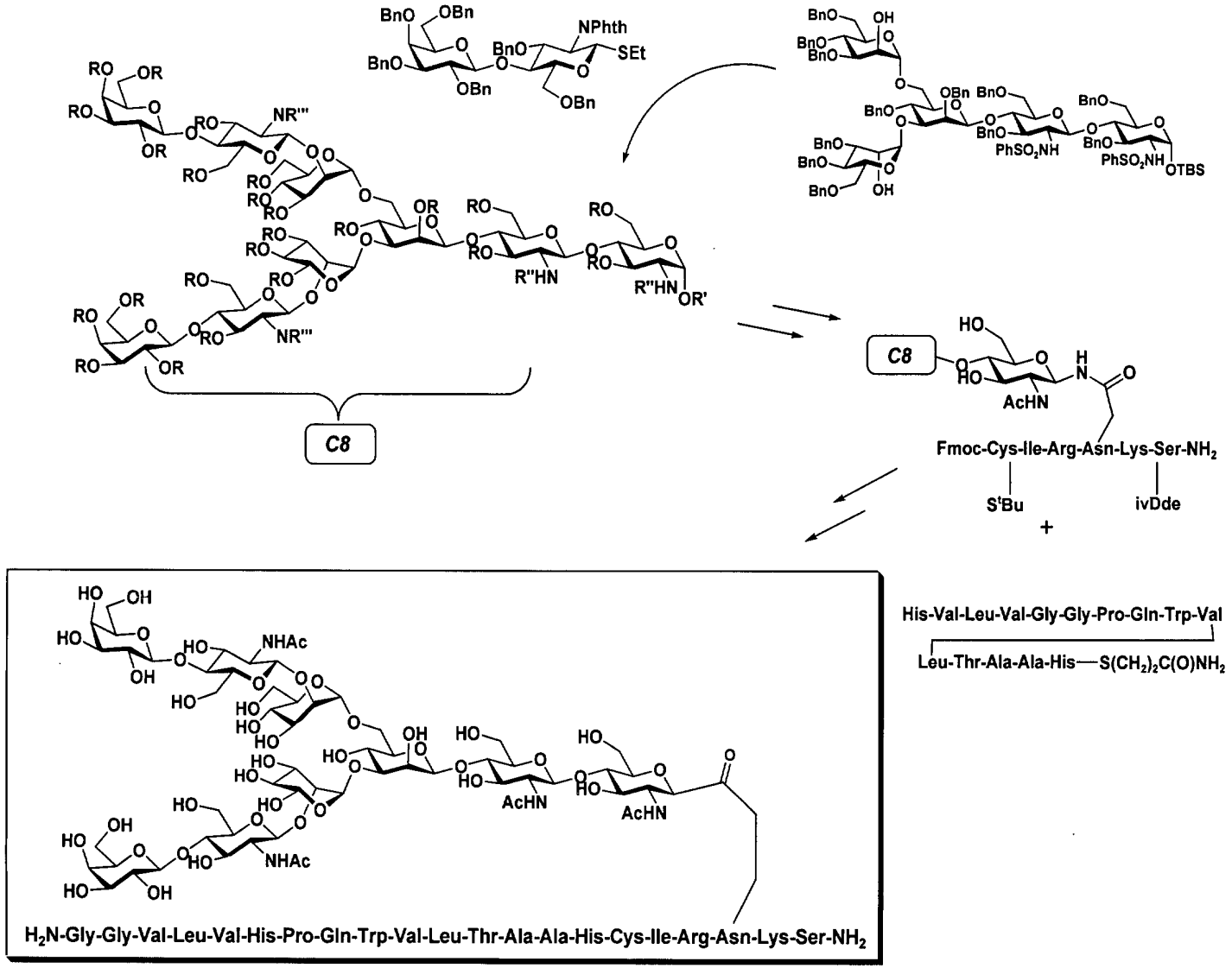
Preparation of selectively deprotected pentasaccharides



Dudkin, Miller, and Danishefsky. submitted to *J Amer Chem Soc*

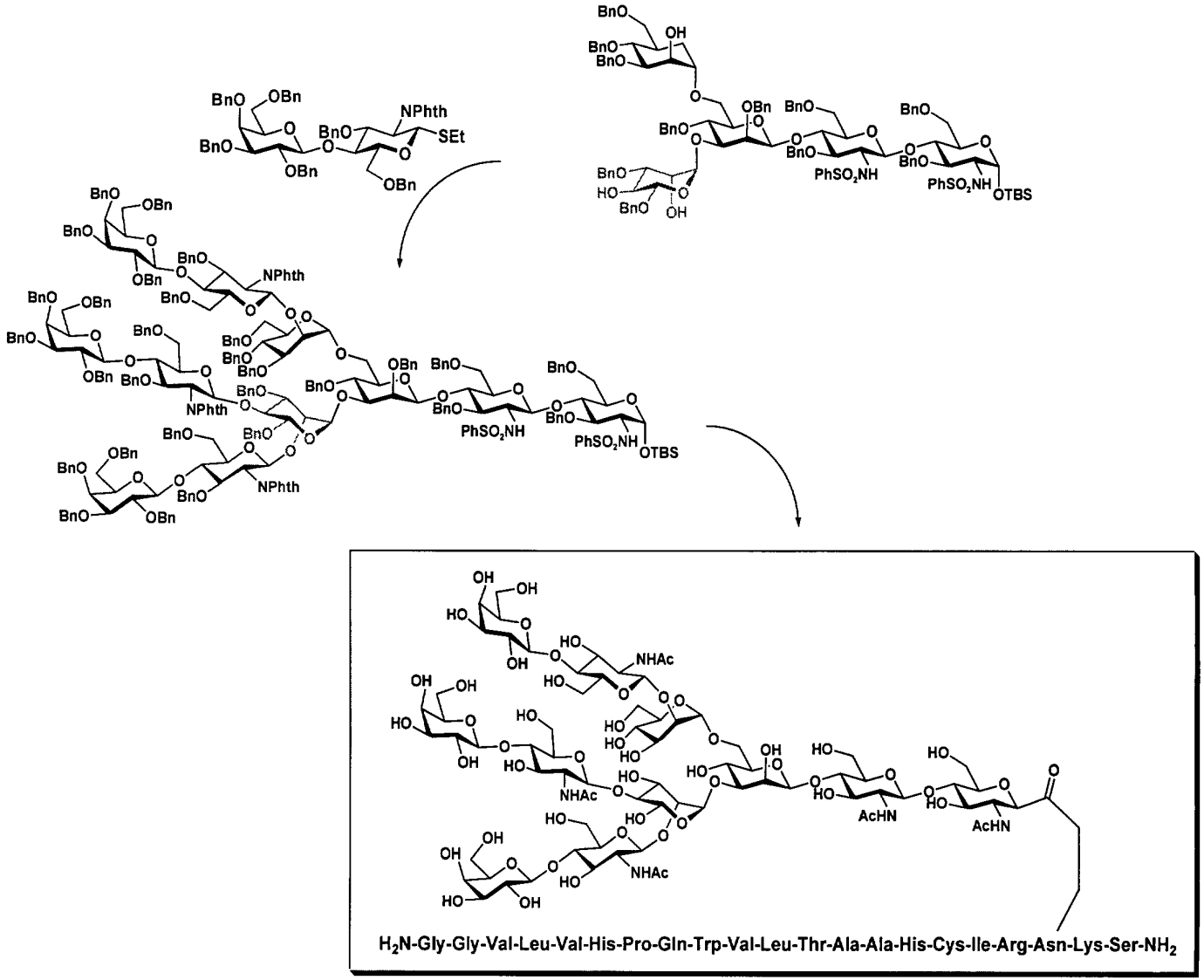
SYNTHESIS OF PSA$^{27-47}$ FRAGMENTS

Preparation of "normal" PSA fragment



Dudkin, Miller, and Danishefsky. submitted to *J Amer Chem Soc*

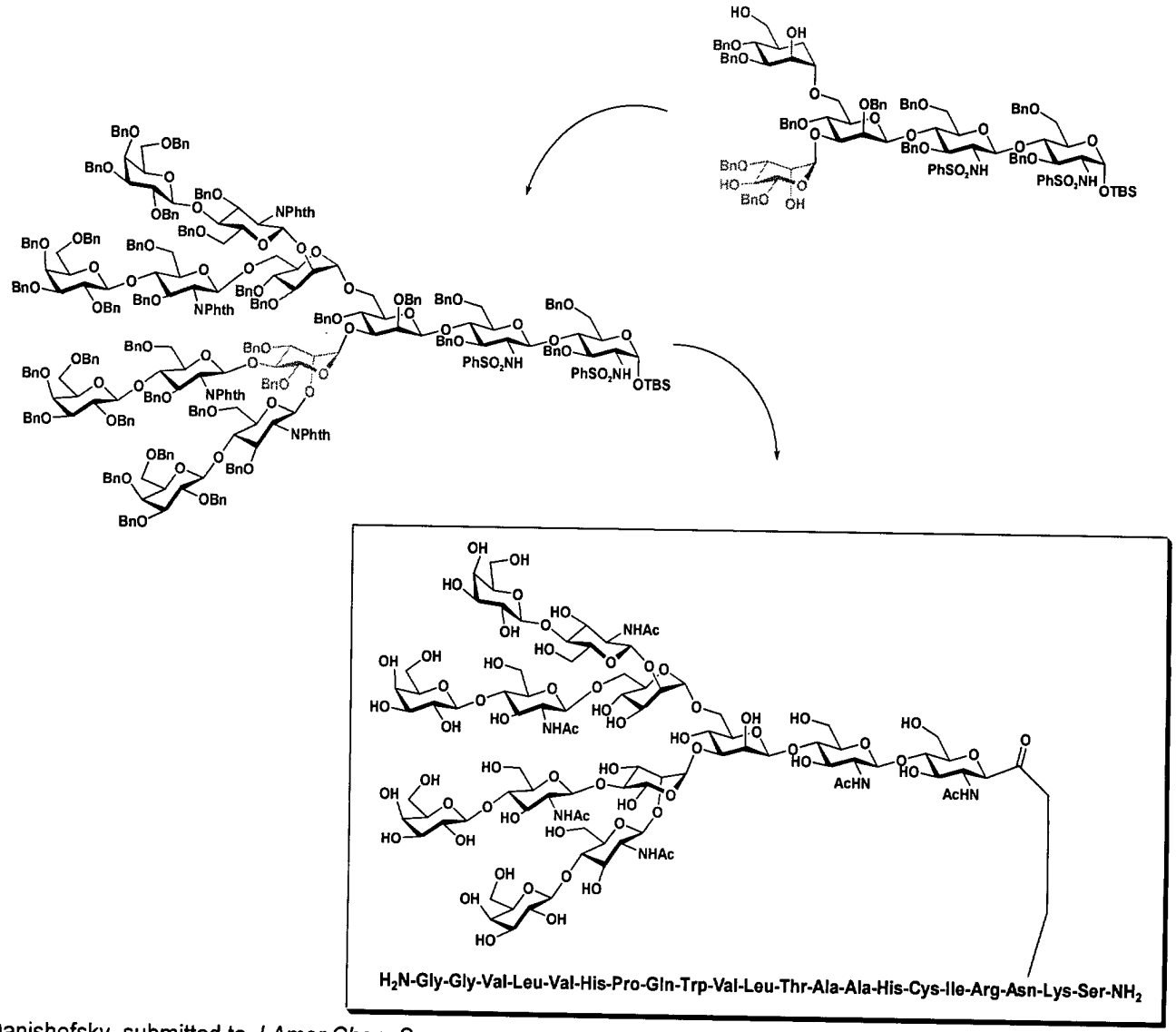
Preparation of tribranched "transformed" PSA fragment



Dudkin, Miller, and Danishefsky. submitted to *J Amer Chem Soc*

WO 2004/050711 PCT/US2003/038471

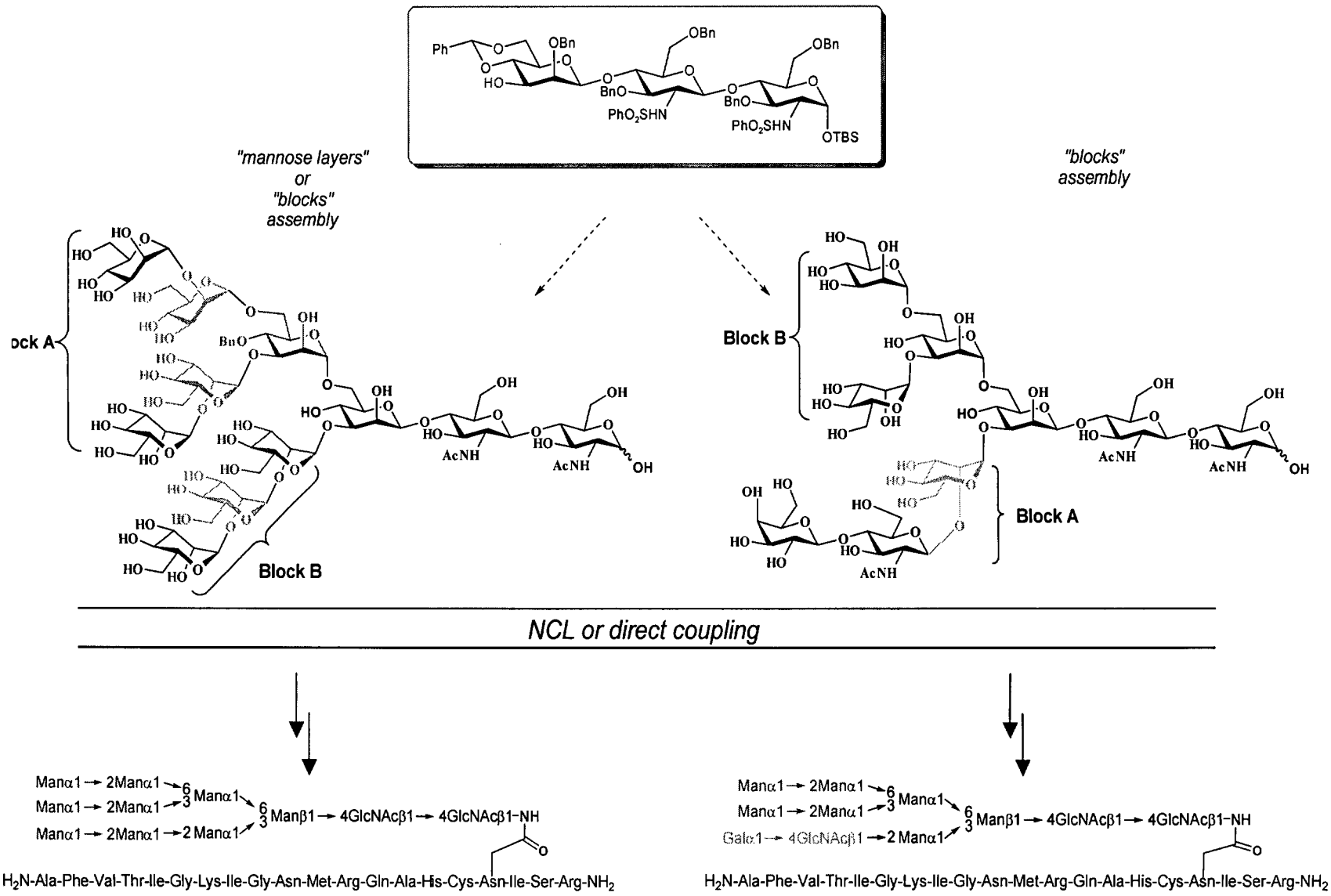
Preparation of tetrabranched "transformed" PSA fragment



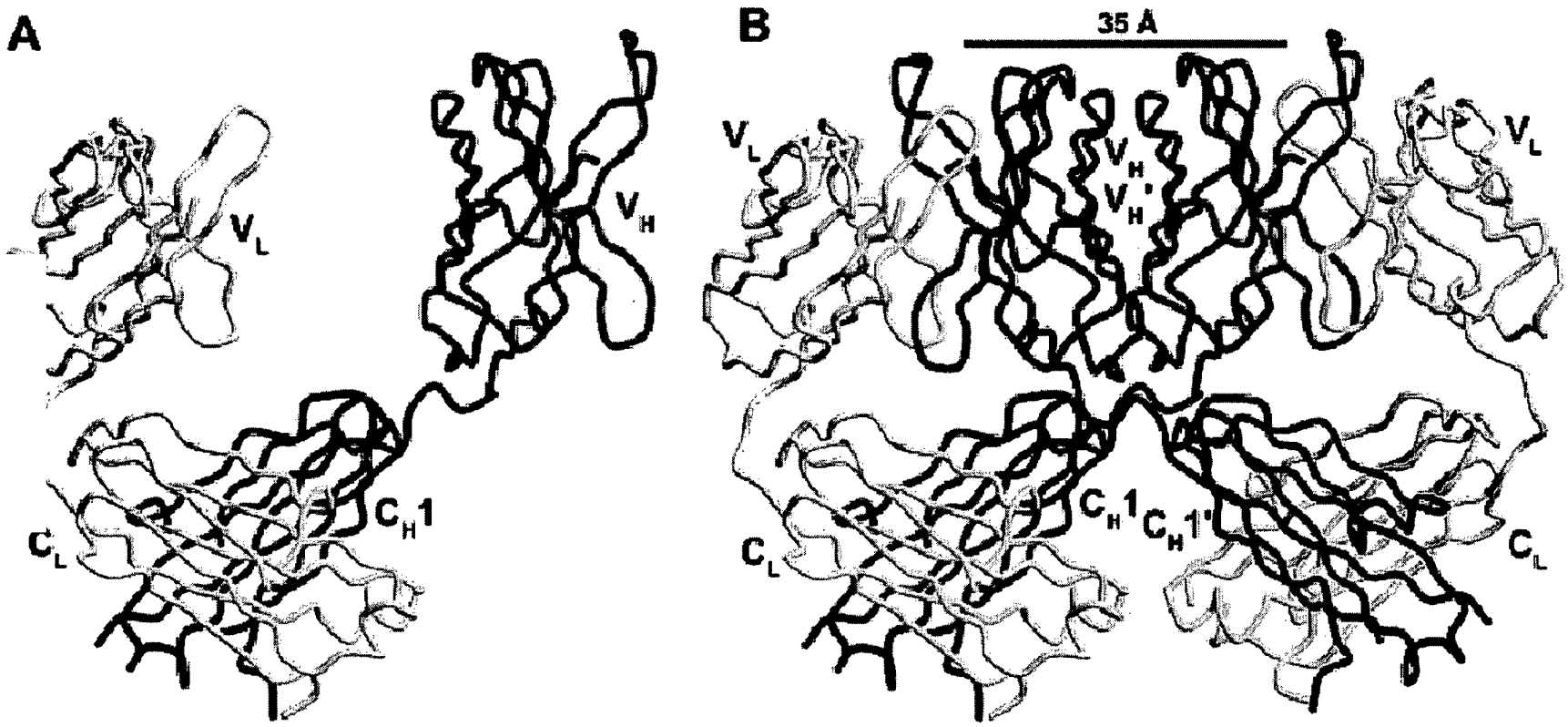
Dudkin, Miller, and Danishefsky. submitted to *J Amer Chem Soc*

WO 2004/050711 PCT/US2003/038471

GENERAL STRATEGY FOR GP120 FRAGMENTS PREPARATION

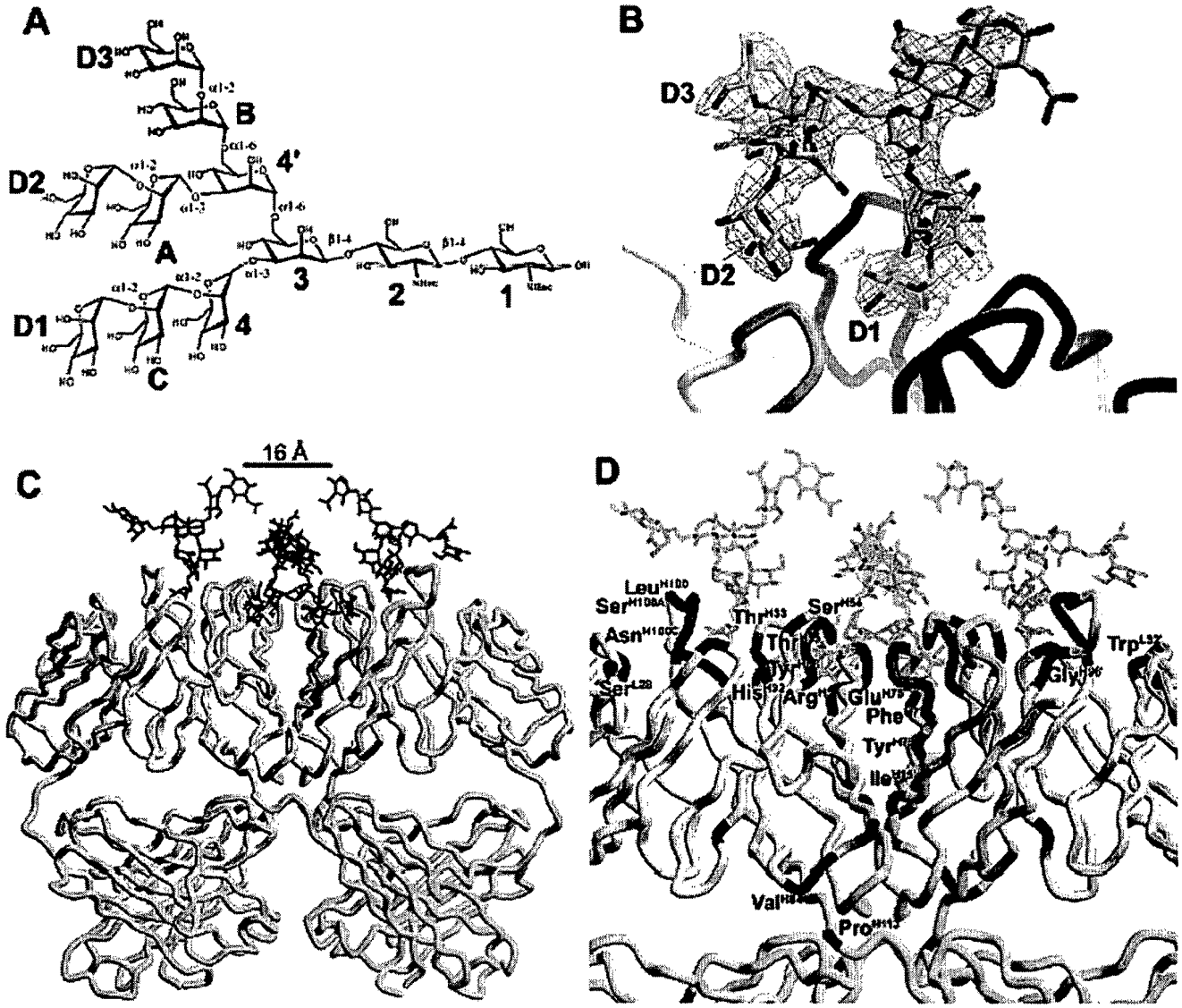


Domain exchange in 2g12 antibody



Calarese et al. *Science* **2003** (300) 2065-2071

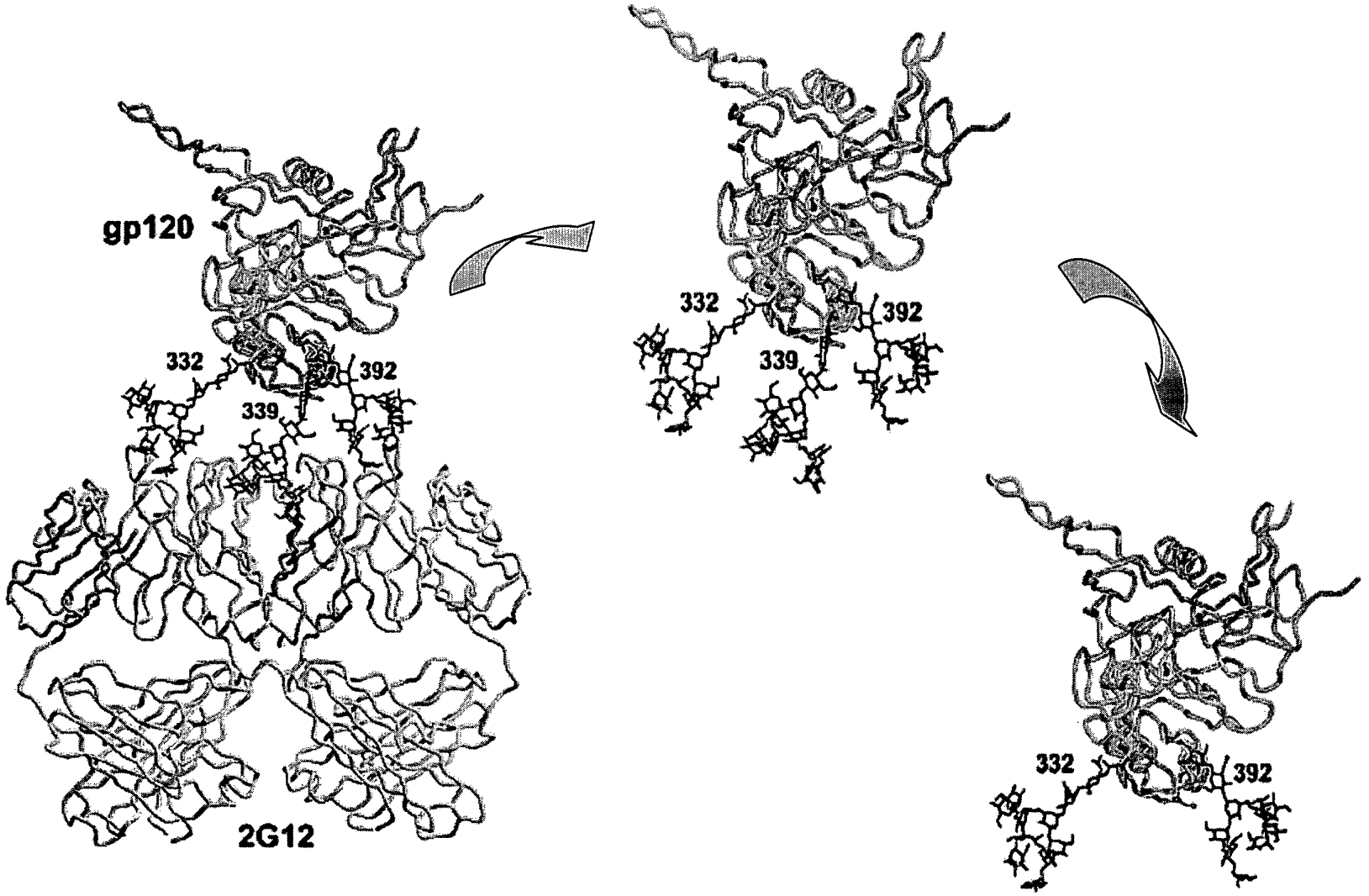
2G12/MAN-9 CRYSTAL STRUCTURE



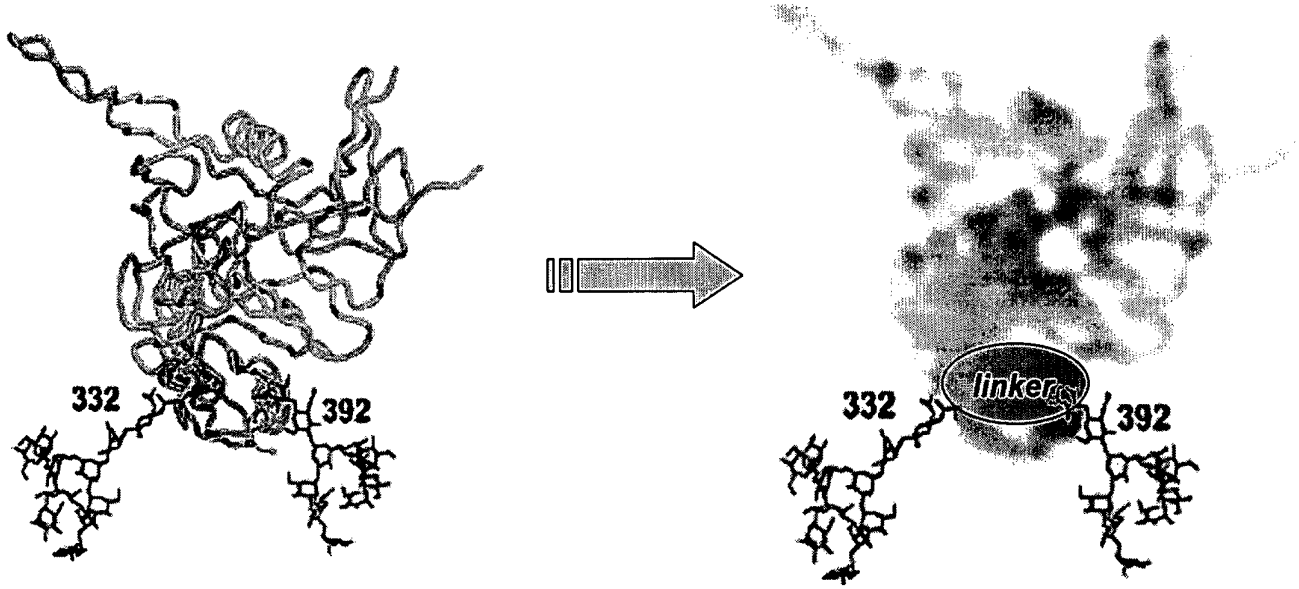
Calarese et al. *Science* **2003** (300) 2065-2071

GLYCAN CLUSTERING: POTENTIAL SOLUTIONS

Second-generation targets: multiple glycans in a single construct



GLYCAN CLUSTERING: POTENTIAL SOLUTIONS



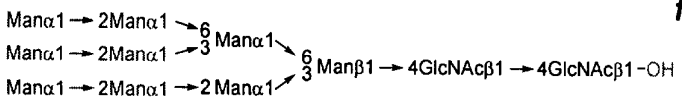
Mimicking a clustered epitope:

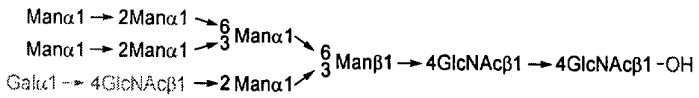
- *Synthesis of a real glycopeptide fragment*
- *Placing glycans on an "artificial" linker system*
- *Multiple conjugation to a carrier protein*

CURRENTLY AVAILABLE FRAGMENTS

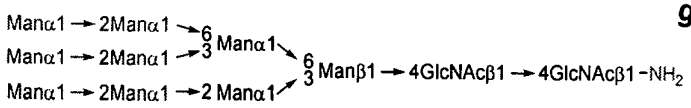
type: high mannose hybrid

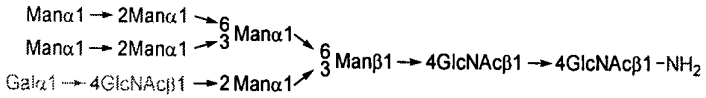
free glycans



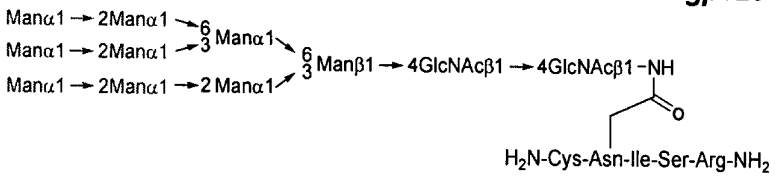


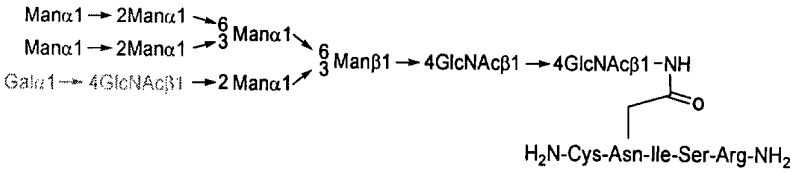
glycosylamines



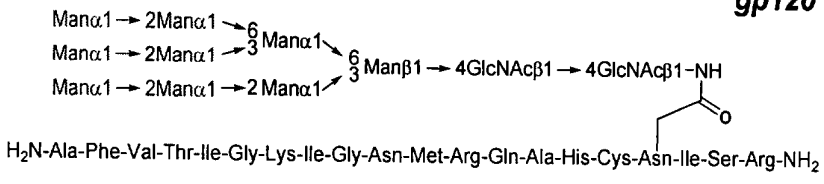


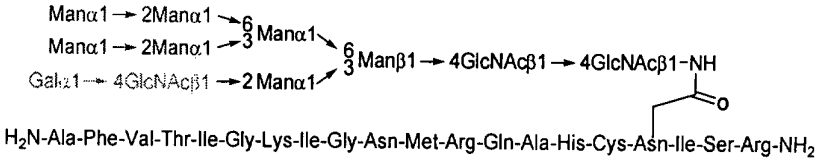
$gp120^{331-335}$ fragments





$gp120^{316-335}$ fragments





SECOND GENERATION TARGETS

1. Multiple monoglycosylated fragments/ carrier protein conjugates

 available immediately

2. Gp120 fragments with multiple glycosylation/ carrier protein

 potentially requires significant synthetic effort

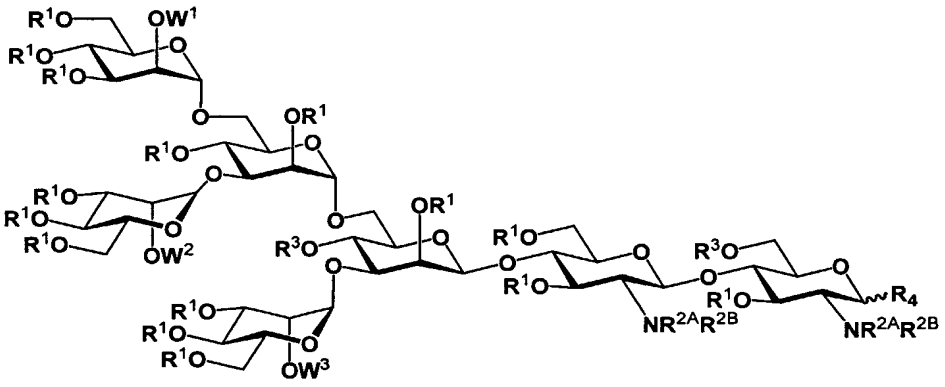
 Multiple glycans on a linker system (polypeptide or non-peptide)/ carrier protein

 may be easily accessible
 potential solution to building a clustered epitope

CLAIMS

What is claimed is:

1. An isolated compound having the structure:

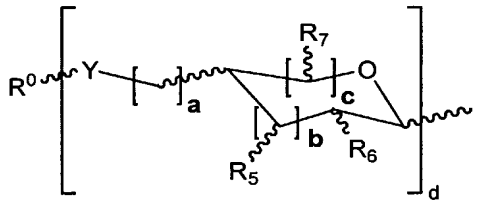


(I)

wherein each occurrence of R^1 is independently hydrogen or an oxygen protecting group;

each occurrence of R^{2A} and R^{2B} is independently hydrogen or a nitrogen protecting group;

each occurrence of R^3 is independently hydrogen, a protecting group or a carbohydrate domain comprising a saccharide moiety having the structure:



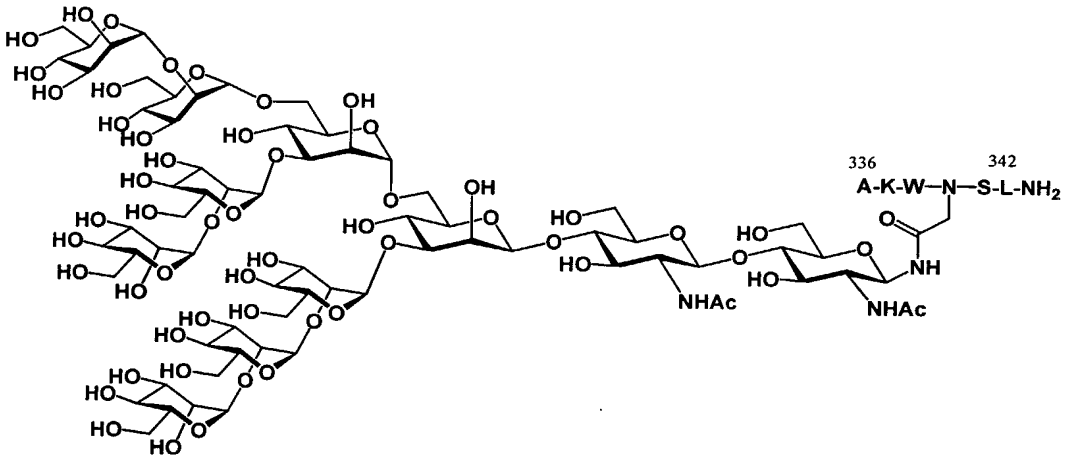
wherein Y is NH or O; wherein a, b and c are each independently 0, 1 or 2; d is an integer from 1-3; with the proviso that the d bracketed structure represents a furanose or pyranose moiety and the sum of b and c is 1 or 2; wherein R^0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein each occurrence of R^5, R^6 and R^7 is independently hydrogen, OH, OR^i, $NR^{ii}R^{iii}$, $NHCOR^i$, F, CH_2OH, CH_2OR^i, or a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of R^i, R^{ii} and R^{iii} is independently hydrogen, a protecting group, a sialic acid moiety, CHO, $COOR^{iv}$, or a substituted or

unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group, or R^{ii} and R^{iii}, taken together with the nitrogen atom to which they are attached, form a substituted or unsubstituted heterocyclic or heteroaryl moiety; and wherein each occurrence of R^{iv} is independently H, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group;

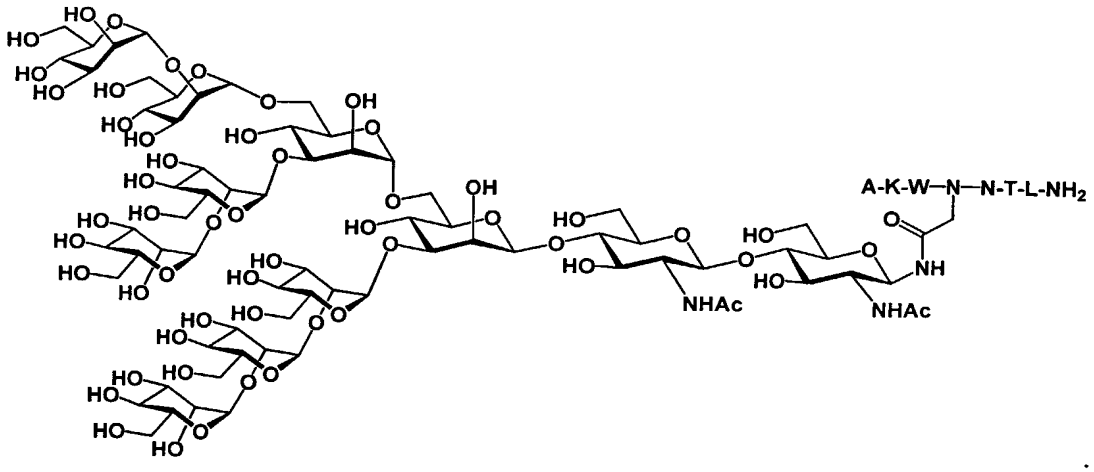
W^1, W^2 and W^3 are independently optionally substituted mannose, galactose or lactosamine moieties;

and wherein R^4 is $-OR^{4A}$ or $-NHR^{4A}$; wherein R^{4A} is hydrogen, aliphatic, heteroaliphatic, aryl, heteroaryl, an amino acyl moiety, an amino acyl residue of a peptide, an amino acyl residue of a protein, or R^{4A} comprises a protein, peptide or lipid moiety covalently linked to the rest of the construct, or to the N or O atom to which it is attached, either directly or through a crosslinker;

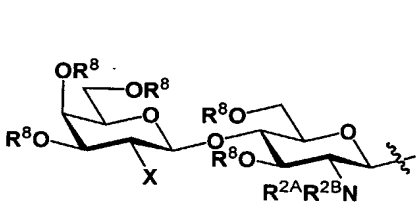
with the proviso that the compound is not a naturally occurring gp120 glycoprotein or a compound having the structure:



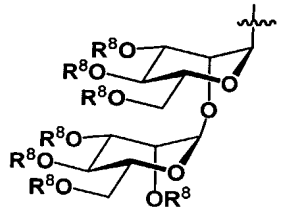
or



2. The compound of claim 1, wherein W^3 is R^1, R^3, as defined above, or a moiety having the structure:

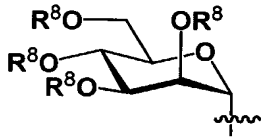


or



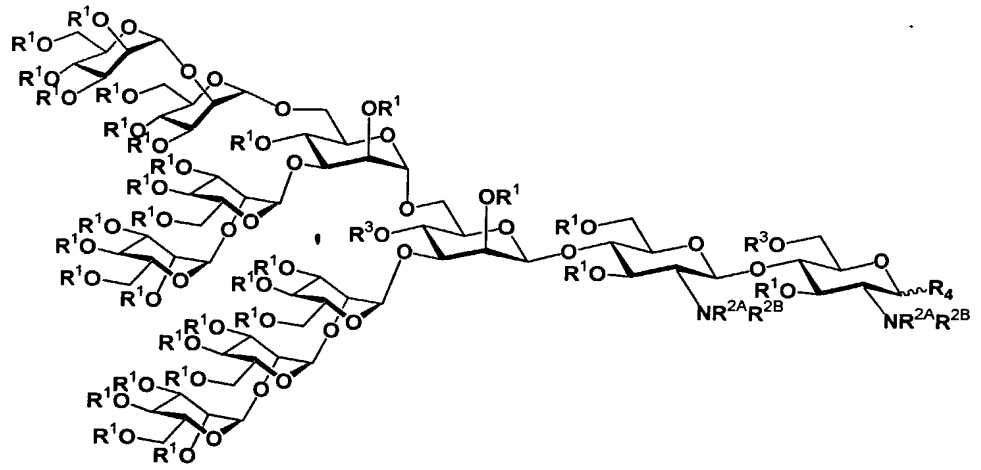
wherein X is $-OR^1$ or $-NR^{2A}R^{2B}$; and each occurrence of R^8 is independently R^1 or a sialic acid moiety.

3. The compound of claim 1, wherein W^1 and W^2 are independently R^1, R^3 or a moiety having the structure:

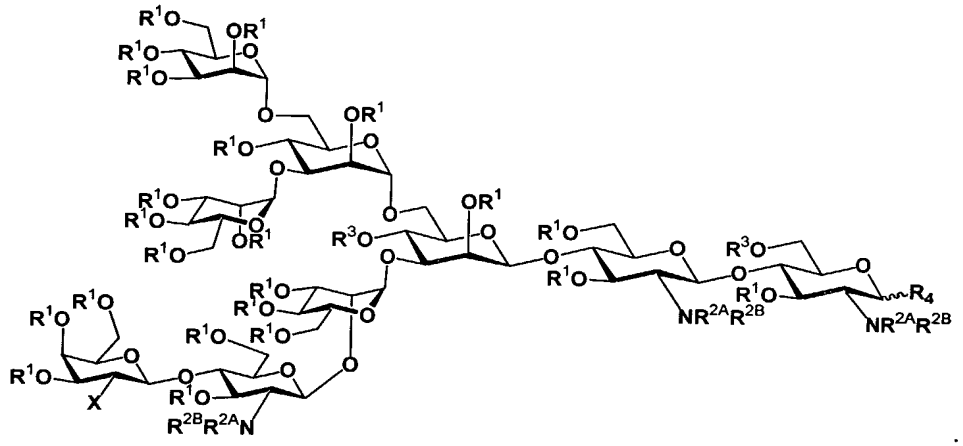


wherein each occurrence of R^8 is independently R^1 or a sialic acid moiety.

4. The compound of claim 1 having the structure:



5. The compound of claim 1 having the structure:



;

wherein X is OR^1 or $NR^{2A}R^{2B}$.

6. The compound of claim 1, 4 or 5, wherein each occurrence of R^1 is independently hydrogen, alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, aryl, heteroaryl, alkylaryl, alkylheteroaryl, $-Si(R^{1A})_3$, $-C(=O)R^{1A}$, $-C(=S)R^{1A}$, $-C(=NR^{1A})R^{1B}$, $-SO_2R^{1A}$, wherein R^{1A} and R^{1B} are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl, heteroaliphatic, heteroalicyclic, aryl, heteroaryl, $-C(=O)R^{1C}$ or $-ZR^{1C}$, wherein Z is –O-, -S-, $-NR^{1D}$, wherein each occurrence of R^{1C} and R^{1D} is independently hydrogen, or an alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocycloalkyl,

heterocycloalkenyl, heterocycloalkynyl, heteroaliphatic, heteroalicyclic, aryl or heteroaryl moiety.

7. The compound of claim 6, wherein each occurrence of R^1 is independently hydrogen, alkylaryl, $-Si(R^{1A})_3$ or $-C(=O)R^{1A}$.

8. The compound of claim 7, wherein each occurrence of R^1 is independently hydrogen, Bn or Bz.

9. The compound of claim 1, 4 or 5, wherein for each occurrence of $-NR^{2A}R^{2B}$, at least one occurrence of R^{2A} or R^{2B} is independently a nitrogen protecting group.

10. The compound of claim 1, 4 or 5, wherein each occurrence of $NR^{2A}R^{2B}$, R^{2A} and R^{2B} is independently hydrogen, alkyl, alkenyl, $-C(=O)R^{2C}$, $-C(=O)OR^{2C}$, $-SR^{2C}$, SO_2R^{2C}, or R^{2A} and R^{2B}, taken together with the nitrogen atom to which they are attached, form a substituted or unsubstituted heterocyclic or heteroaryl moiety; wherein each occurrence of R^{2C} is independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl, heteroaliphatic, heteroalicyclic, aryl, heteroaryl, $-C(=O)R^{2D}$ or $-ZR^{2D}$, wherein Z is –O-, -S-, $-NR^{2E}$, wherein each occurrence of R^{2D} and R^{2E} is independently hydrogen, or an alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl, heteroaliphatic, heteroalicyclic, aryl or heteroaryl moiety.

11. The compound of claim 1, 4 or 5, wherein for each occurrence of $-NR^{2A}R^{2B}$, at least one occurrence of R^{2A} or R^{2B} is independently $-C(=O)R^{2A}$ or SO_2R^{2A}; or R^{2A} and R^{2B}, taken together with the nitrogen atom to which they are attached, form a substituted or unsubstituted heterocyclic or heteroaryl moiety.

12. The compound of claim 11, wherein for each occurrence of $-NR^{2A}R^{2B}$, at least one occurrence of R^{2A} or R^{2B} is independently acyl, $-SO_2Ph$ or R^{2A} and R^{2B},

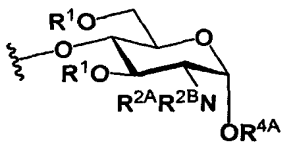
taken together with the nitrogen atom to which they are attached, form an azide or a substituted or unsubstituted phthalimide moiety.

13. The compound of claim 5, wherein X is $-OR^1$:

14. The compound of claim 1, wherein each occurrence of R^3 is independently hydrogen, alkylaryl, $-Si(R^{3A})_3$ or $-C(=O)R^{3A}$, wherein R^{3A} is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl, heteroaliphatic, heteroalicyclic, aryl, heteroaryl, $-C(=O)R^{3B}$ or $-ZR^{3B}$, wherein Z is –O-, -S-, $-NR^{3C}$, wherein each occurrence of R^{3B} and R^{3C} is independently hydrogen, or an alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl, heteroaliphatic, heteroalicyclic, aryl or heteroaryl moiety.

15. The compound of claim 1, 4 or 5, wherein each occurrence R^1 and R^3 is independently hydrogen, alkylaryl, $-Si(R^{3A})_3$ or $-C(=O)R^{3A}$, wherein R^{3A} is hydrogen, alkylaryl, $-Si(R^{3A})_3$ or $-C(=O)R^{3A}$, wherein R^{3A} is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl, heteroaliphatic, heteroalicyclic, aryl, heteroaryl, $-C(=O)R^{3B}$ or $-ZR^{3B}$, wherein Z is –O-, -S-, $-NR^{3C}$, wherein each occurrence of R^{3B} and R^{3C} is independently hydrogen, or an alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl, heteroaliphatic, heteroalicyclic, aryl or heteroaryl moiety.

16. The compound of claim 1, 4 or 5, wherein R^4 is $-OR^{4A}$ and the saccharide unit bearing R^4 has the structure:



wherein R^1, R^{2A} and R^{2B} are as defined generally above and in classes and subclasses herein; R^{4A} is hydrogen, alkyl, alkenyl, alkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, aryl, heteroaryl, alkylaryl, alkylheteroaryl, an amino acyl moiety, an amino acyl residue of a peptide, an amino acyl residue of a protein, -$Si(R^{4B})_3$, -$C(=O)R^{4B}$, -$C(=S)R^{4B}$, -$C(=NR^{4B})R^{4C}$, -SO_2R^{4B}, wherein R^{4B} and R^{4C} are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl, heteroaliphatic, heteroalicyclic, aryl, heteroaryl, -$C(=O)R^{4D}$ or –ZR^{4D}, wherein Z is –O-, -S-, -NR^{4E}, wherein each occurrence of R^{4D} and R^{4E} is independently hydrogen, or an alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, heteroalkyl, heteroalkenyl, heteroalkynyl, heterocycloalkyl, heterocycloalkenyl, heterocycloalkynyl, heteroaliphatic, heteroalicyclic, aryl or heteroaryl moiety; or R^{4A} comprises a protein, peptide or lipid moiety covalently linked to the O atom to which it is attached, either directly or through a crosslinker.

17. The compound of claim 1, 4 or 5, wherein R^4 is –NHR^{4A} and the saccharide unit bearing R^4 has the structure:



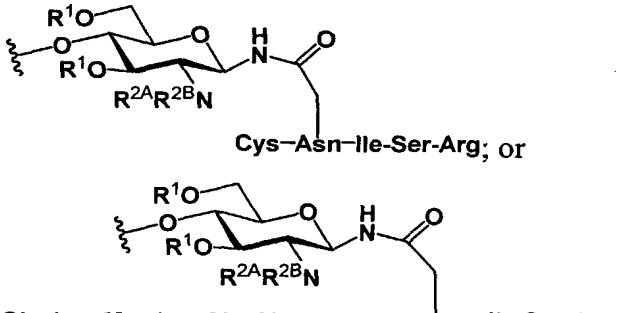
wherein R^1, R^{2A} and R^{2B} are as defined generally above and in classes and subclasses herein; and R^{4A} is hydrogen, aliphatic, heteroaliphatic, aryl, heteroaryl, an amino acyl moiety, an amino acyl residue of a peptide, an amino acyl residue of a protein, or R^{4A} comprises a protein, peptide or lipid moiety covalently linked to the rest of the construct, or to the N atom to which it is attached, either directly or through a crosslinker.

18. The compound of claim 17, wherein R^{4A} is hydrogen.

19. The compound of claim 17, wherein R^{4A} comprises an amino acyl residue of a peptide whose structure is either identical or closely related to that of gp120 near an N-glycosylation site.

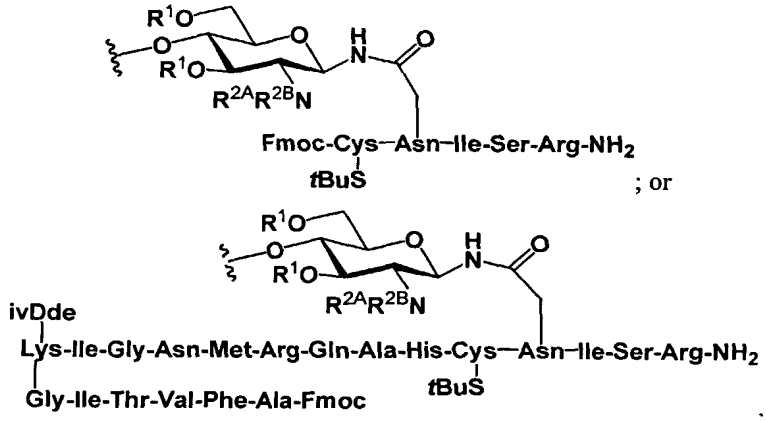
20. The compound of claim 19, wherein the amino acyl residue is Asn.

21. The compound of claim 1, 4 or 5, wherein R^4 is $-NHR^{4A}$ wherein R^{4A} comprises an Asparagine residue (Asn) of a peptide whose structure is either identical or closely related to that of gp120 near an N-glycosylation site and the saccharide unit bearing R^4 has the structure:

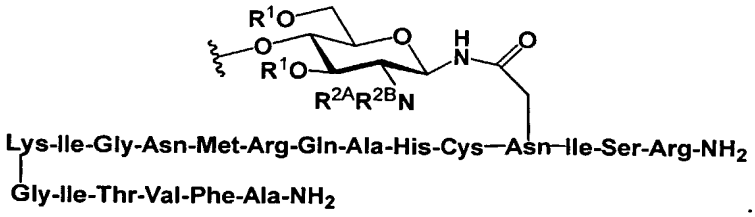


wherein any of the amino acid residues may bear one or more protecting groups.

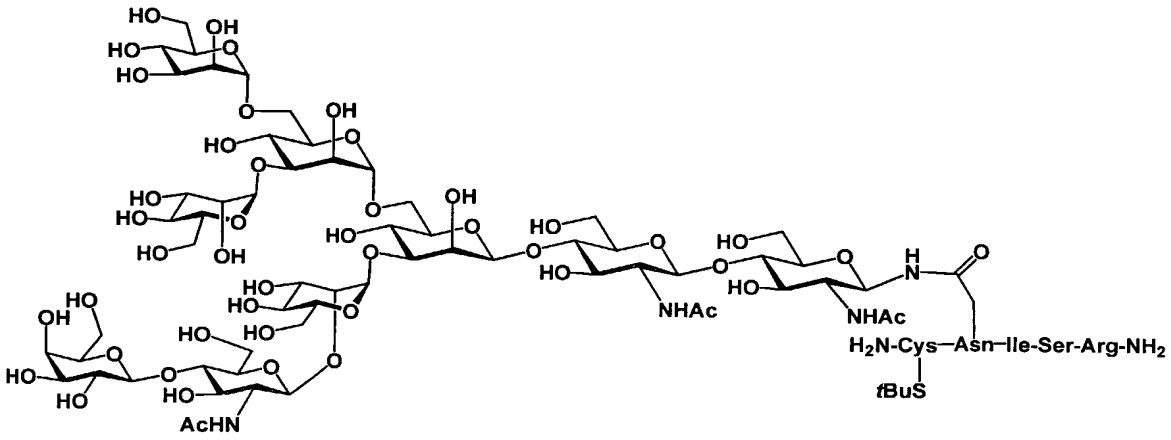
22. The compound of claim 21, wherein the saccharide unit bearing R^4 has the structure:



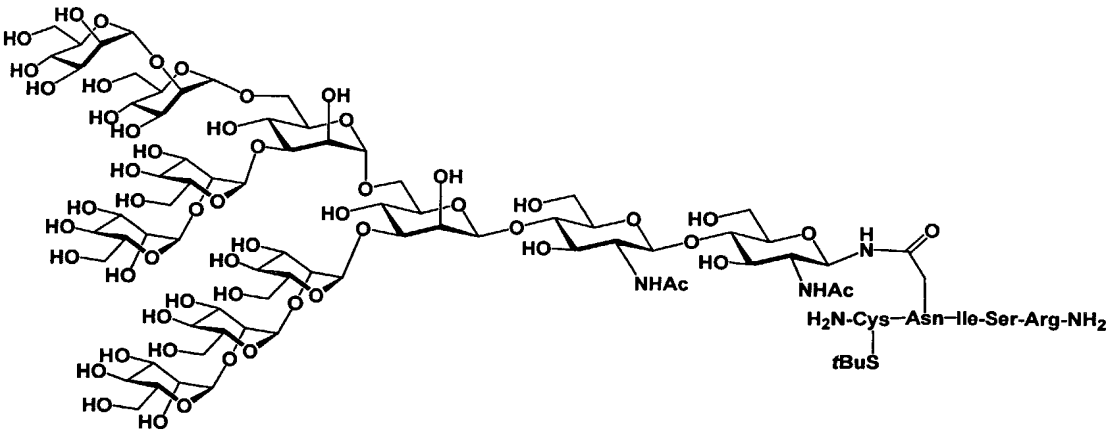
23. The compound of claim 21, wherein the saccharide unit bearing R^4 has the structure:



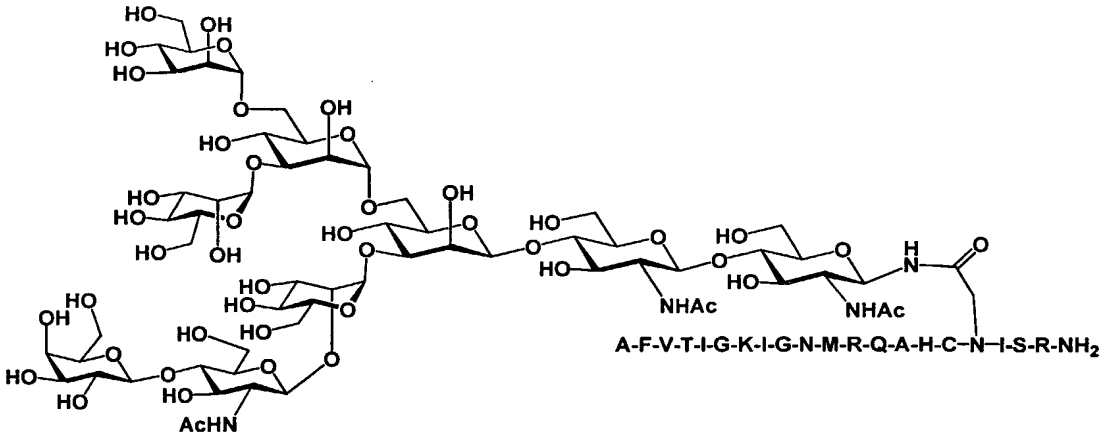
24. The compound of claim 1 having the structure:



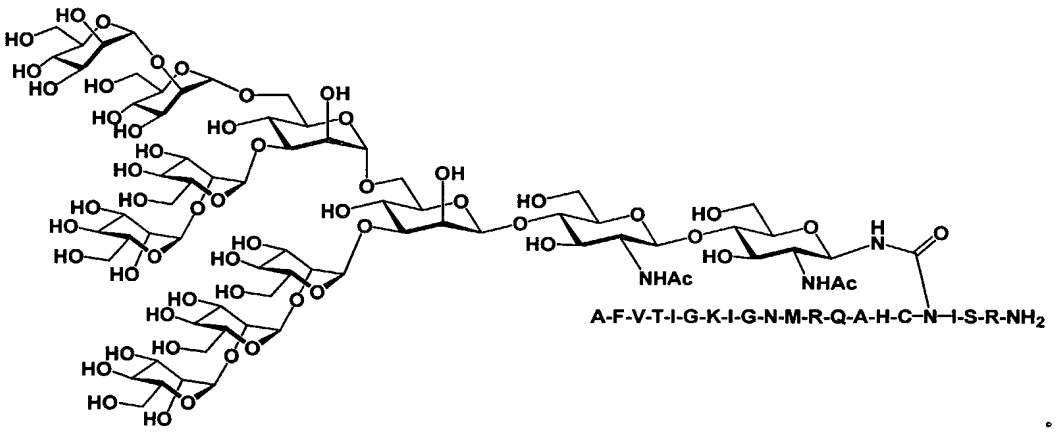
25. The compound of claim 1 having the structure:



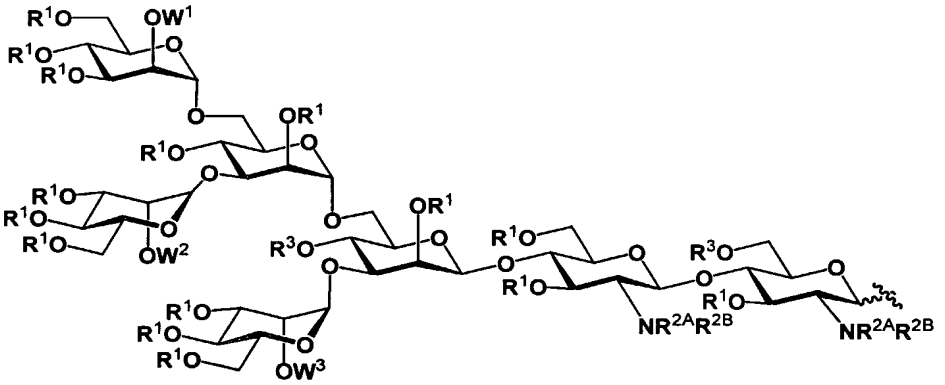
26. The compound of claim 1 having the structure:



27. The compound of claim 1 having the structure:



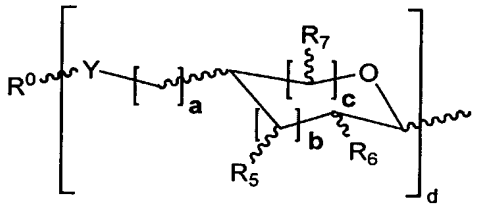
28. A multi-antigenic construct comprising one or more carbohydrate domains having the structure:



wherein each occurrence of R^1 is independently hydrogen or an oxygen protecting group;

each occurrence of R^{2A} and R^{2B} is independently hydrogen or a nitrogen protecting group;

each occurrence of R^3 is independently hydrogen, a protecting group or a carbohydrate domain comprising a saccharide moiety having the structure:

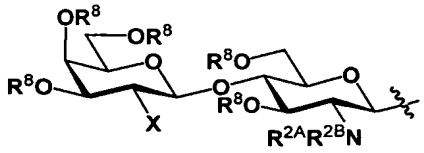


wherein Y is NH or O; wherein a, b and c are each independently 0, 1 or 2; d is an integer from 1-3; with the proviso that the d bracketed structure represents a furanose or pyranose moiety and the sum of b and c is 1 or 2; wherein R^0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein each occurrence of R^5, R^6 and R^7 is independently hydrogen, OH, OR^i, $NR^{ii}R^{iii}$, $NHCOR^i$, F, CH_2OH, CH_2OR^i, or a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of R^i, R^{ii} and R^{iii} is independently hydrogen, a protecting group, a sialic acid moiety, CHO, $COOR^{iv}$, or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group, or R^{ii} and R^{iii}, taken together with the nitrogen atom to which they are attached, form a substituted or unsubstituted heterocyclic or heteroaryl moiety; and wherein each occurrence of R^{iv} is independently H, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group;

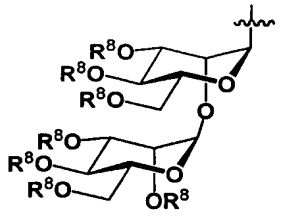
W^1, W^2 and W^3 are independently optionally substituted mannose, galactose or lactosamine moieties;

wherein each carbohydrate domain is independently covalently bound to a linker system, said linker system being a peptide or non-peptide nature, and wherein the linker system may be cyclic or acyclic.

29. The construct of claim 28, wherein W^3 is R^1, R^3, as defined in claim 28, or a moiety having the structure:

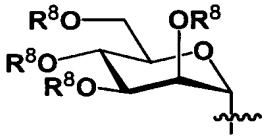


or



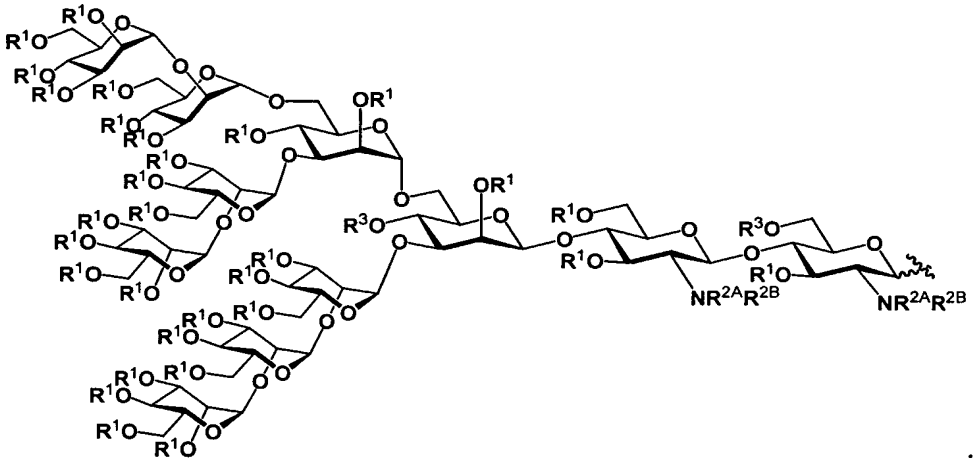
wherein X is $-OR^1$ or $-NR^{2A}R^{2B}$; and each occurrence of R^8 is independently R^1 or a sialic acid moiety.

30. The construct of claim 28, wherein W^1 and W^2 are independently R^1, R^3 or a moiety having the structure:

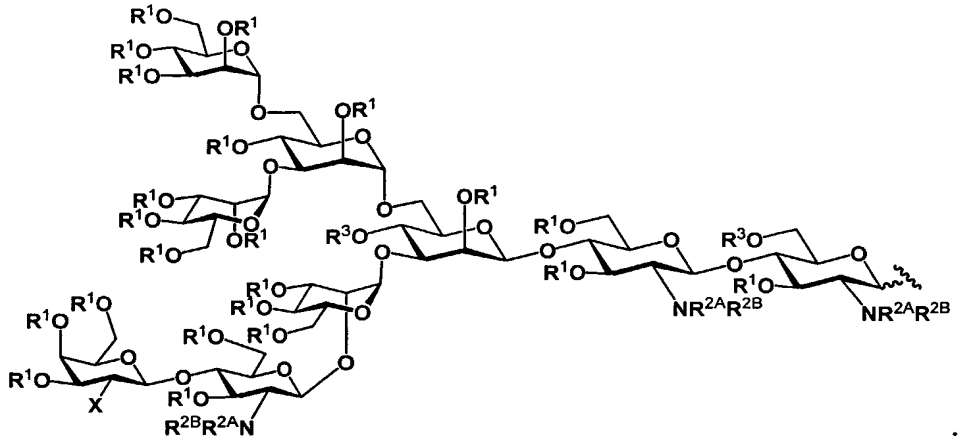


wherein each occurrence of R^8 is independently R^1 or a sialic acid moiety.

31. The construct of claim 28, wherein one or more carbohydrate domains have the structure:



32. The construct of claim 28, wherein one or more carbohydrate domains have the structure:

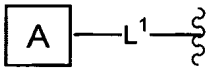


33. The construct of claim 28, wherein the linker system is a peptide.

34. The construct of claim 28, wherein the linker system is designed to approximate the spatial position(s) of carbohydrate(s) in gp120.

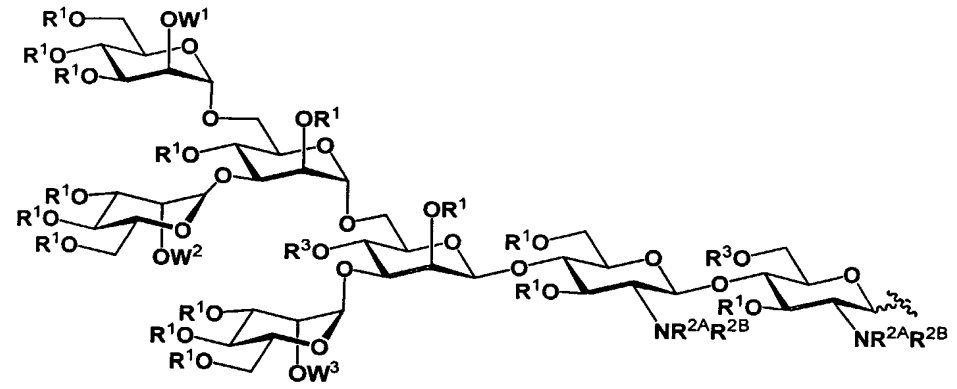
35. The construct of claim 28, wherein the linker system is further attached to a carrier immunostimulant.

36. The multi-antigenic construct of claim 28, wherein the construct comprises a backbone made up of two or more amino acids or other structural units, wherein one or more of said amino acids or structural units is/are independently substituted with a glycosidic moiety having the structure:

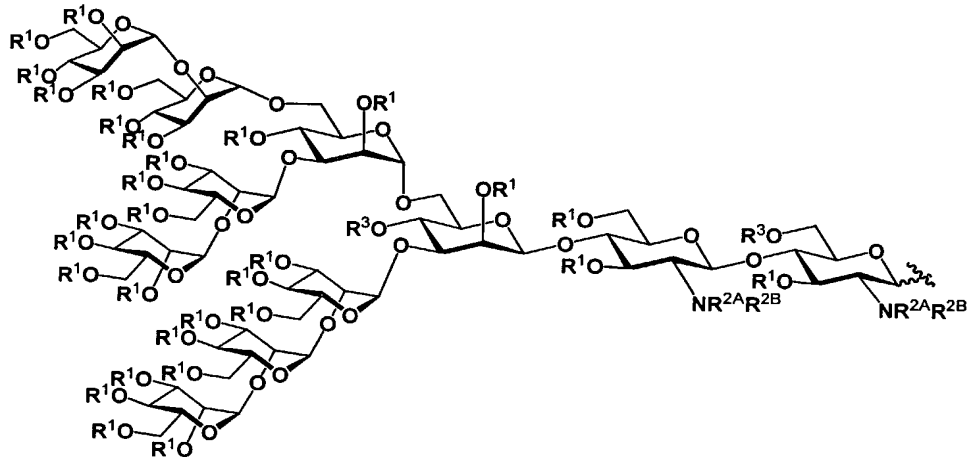


wherein each occurrence of L^1 is independently a substituted or unsubstituted, linear or branched, cyclic or acyclic, saturated or unsaturated aliphatic or heteroaliphatic moiety; and

each occurrence of A is independently a carbohydrate domain of formula:

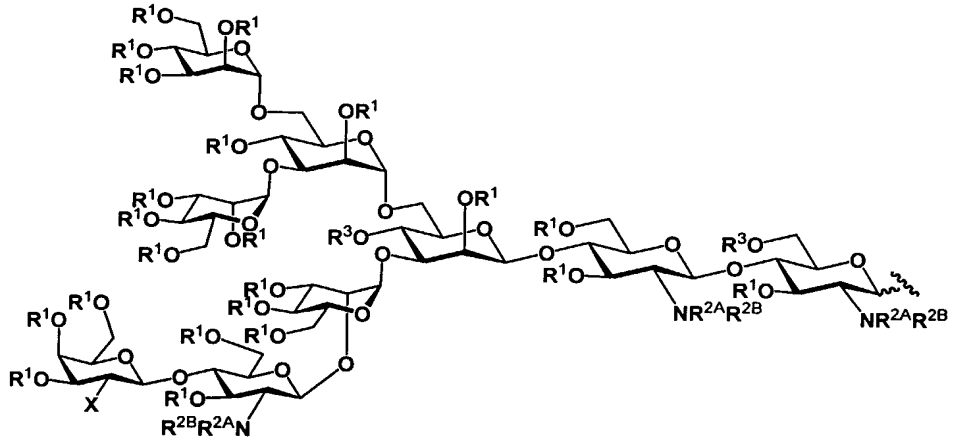


(I^{det})



(II^{det})

or

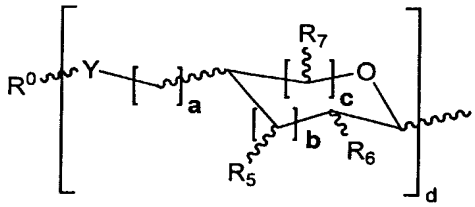


(III^{det})

wherein each occurrence of R^1 is independently hydrogen or an oxygen protecting group;

each occurrence of R^{2A} and R^{2B} is independently hydrogen or a nitrogen protecting group;

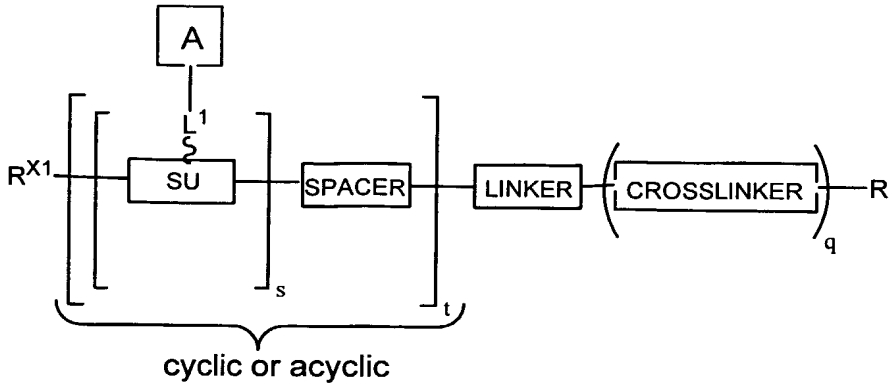
each occurrence of R^3 is independently hydrogen, a protecting group or a carbohydrate domain comprising a saccharide moiety having the structure:



wherein Y is NH or O; wherein a, b and c are each independently 0, 1 or 2; d is an integer from 1-3; with the proviso that the d bracketed structure represents a furanose or pyranose moiety and the sum of b and c is 1 or 2; wherein R^0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein each occurrence of R^5, R^6 and R^7 is independently hydrogen, OH, OR^i, $NR^{ii}R^{iii}$, $NHCOR^i$, F, CH_2OH, CH_2OR^i, or a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of R^i, R^{ii} and R^{iii} is independently hydrogen, a protecting group, a sialic acid moiety, CHO, $COOR^{iv}$, or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group, or R^{ii} and R^{iii}, taken together with the nitrogen atom to which they are attached, form a substituted or unsubstituted heterocyclic or heteroaryl moiety; and wherein each occurrence of R^{iv} is independently H, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group;

W^1, W^2 and W^3 are independently optionally substituted mannose, galactose or lactosamine moieties.

37. The construct of claim 36 having the structure:



wherein q is 0 or 1;

each occurrence of s is independently an integer from 2-20;

t is an integer from 1-6;

R^{X1} is hydrogen, alkyl, acyl, aryl, heteroaryl, -alkyl(aryl), -alkyl(heteroaryl) or a nitrogen protecting group; or R^{X1} is covalently bound to a substituent on the last occurrence of the spacer, thereby forming a cyclic backbone;

R is hydrogen or an immunogenic carrier;

each occurrence of the structural unit SU is independently a substituted or unsubstituted aliphatic, heteroaliphatic, aryl, heteroaryl or peptidic moiety;

each occurrence of the spacer is independently a substituted or unsubstituted aliphatic, heteroaliphatic, aryl, heteroaryl or peptidic moiety;

the linker is either a free carboxylic acid, –O-, (carboxamido)alkyl carboxamide, MBS, primary carboxamide, mono- or dialkyl carboxamide, mono- or diarylcarboxamide, linear or branched chain (carboxy)alkyl carboxamide, linear or branched chain (alkoxycarbonyl)alkyl-carboxamide, linear or branched chain (carboxy)arylalkylcarboxamide, linear or branched chain (alkoxycarbonyl)alkylcarboxamide, an oligoester fragment comprising from 2 to about 20 hydroxy acyl residues, a peptidic fragment comprising from 2 to about 20 amino acyl residues, or a linear or branched chain alkyl or aryl carboxylic ester;

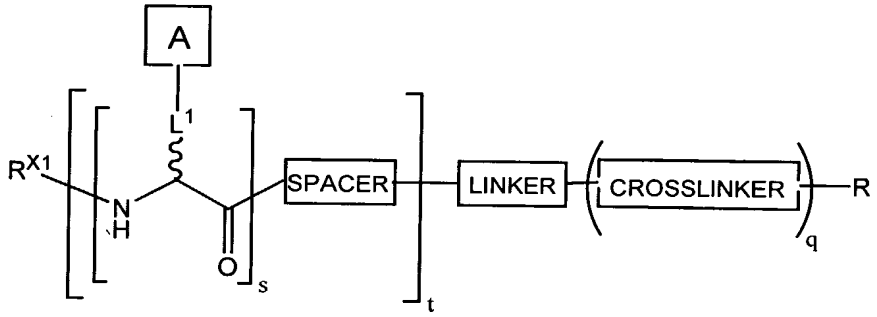
each occurrence of L^1 is independently a substituted or unsubstituted aliphatic or heteroaliphatic moiety; and

each occurrence of A is independently a carbohydrate domain of formula **(I^{det}), (II^{det})** or **(III^{det})**.

38. The construct of claim 37, wherein each occurrence of L^1 is independently $-O(CHR^{aa})_n-$ or $-NHC(=O)(CHR^{aa})_n-$ wherein each occurrence of n is independently an integer from 1-10; and each occurrence of R^{aa} is hydrogen, lower alkyl, aryl, heteroaryl, -alkyl(aryl) or –alkyl(heteroaryl).

39. The construct of claim 37, the structural unit SU, for each occurrence, is independently an amino acid residue, a peptidyl moiety, a bivalent aryl or heteroaryl moiety or a substituted or unsubstituted C_{1-6}alkylidene or C_{2-6}alkenylidene chain wherein up to two non-adjacent methylene units are independently optionally replaced by CO, CO_2, COCO, $CONR^{Z1}$, $OCONR^{Z1}$, $NR^{Z1}NR^{Z2}$, $NR^{Z1}NR^{Z2}CO$, $NR^{Z1}CO$, $NR^{Z1}CO_2$, $NR^{Z1}CONR^{Z2}$, SO, SO_2, $NR^{Z1}SO_2$, SO_2NR^{Z1}, $NR^{Z1}SO_2NR^{Z2}$, O, S, or NR^{Z1}; wherein each occurrence of R^{Z1} and R^{Z2} is independently hydrogen, alkyl, heteroalkyl, aryl, heteroaryl or acyl.

40. The construct of claim 39, wherein each occurrence of the structural unit SU is an amino acid residue, and the clustered multi-antigenic construct has the structure:



wherein q is 0 or 1;

each occurrence of s is independently an integer from 2-20;

t is an integer from 1-6;

R^{X1} is hydrogen, alkyl, acyl, aryl, heteroaryl, -alkyl(aryl), -alkyl(heteroaryl) or a nitrogen protecting group;

R is hydrogen or an immunogenic carrier;

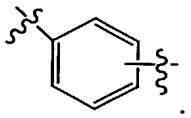
each occurrence of the spacer is independently a substituted or unsubstituted aliphatic, heteroaliphatic, aryl, heteroaryl or peptidic moiety;

the linker is either a free carboxylic acid, –O-, (carboxamido)alkyl carboxamide, MBS, primary carboxamide, mono- or dialkyl carboxamide, mono- or diarylcarboxamide, linear or branched chain (carboxy)alkyl carboxamide, linear or branched chain (alkoxycarbonyl)alkyl-carboxamide, linear or branched chain (carboxy)arylalkylcarboxamide, linear or branched chain (alkoxycarbonyl)alkylcarboxamide, an oligoester fragment comprising from 2 to about 20 hydroxy acyl residues, a peptidic fragment comprising from 2 to about 20 amino acyl residues, or a linear or branched chain alkyl or aryl carboxylic ester;

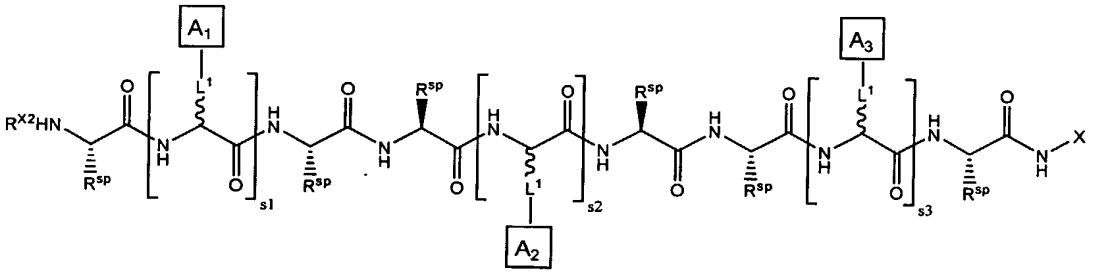
each occurrence of L^1 is independently a substituted or unsubstituted aliphatic or heteroaliphatic moiety; and

each occurrence of A is independently a carbohydrate domain of formula **(I^{det}), (II^{det})** or **(III^{det})**.

41. The construct of claim 37 or 40, wherein the spacer, for each occurrence, is independently $-(CHR^{sp})_n-$, where n is 1-8 and each occurrence of R^{sp} is independently hydrogen, alkyl, cycloalkyl, aryl, heteroaryl, -alkyl(aryl), -alkyl(heteroaryl), $-OR^{sp1}$, $-SR^{sp}1$ or $-NR^{sp1}R^{sp2}$ where R^{sp1} and R^{sp1} are independently hydrogen or lower alkyl; a peptidyl moiety comprising one or more α-amino acid residues, or a bivalent aryl moiety having the structure:



42. The construct of claim 40 having the structure:



wherein each occurrence of L^1 is independently $-O(CHR^{aa})_n-$ or –

$NHC(=O)(CHR^{aa})_n$- wherein each occurrence of n is independently an integer from 1-10; and each occurrence of R^{aa} is hydrogen, lower alkyl, aryl, heteroaryl, -alkyl(aryl) or –alkyl(heteroaryl);

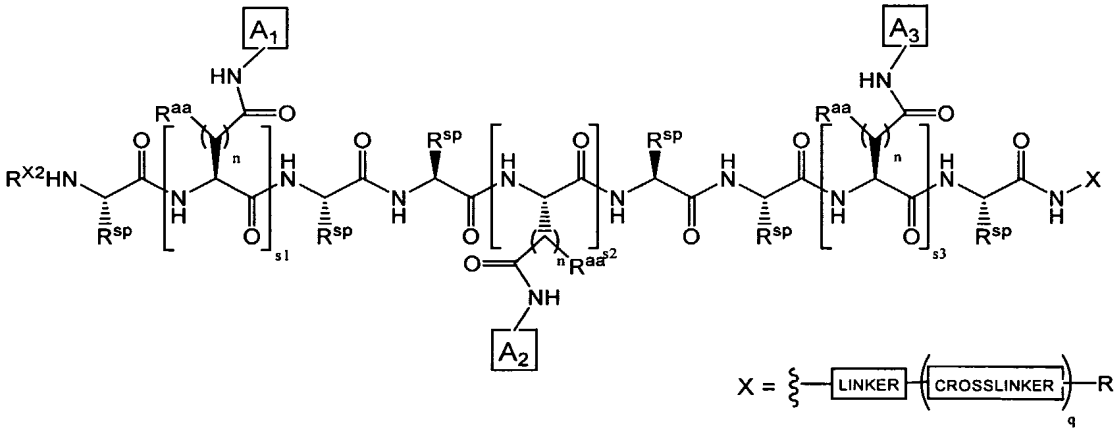
each occurrence of R^{sp} is independently hydrogen, alkyl, cycloalkyl, aryl, heteroaryl, -alkyl(aryl), -alkyl(heteroaryl), $-OR^{sp1}$, $-SR^{sp}1$ or $-NR^{sp1}R^{sp2}$ where R^{sp1} and R^{sp1} are independently hydrogen or lower alkyl; or a peptidyl moiety comprising one or more α-amino acid residues;

s1, s2 and s3 are independently integers from 2-5;

A_1-A_3 are independently a carbohydrate domain of formula **(I^{det})**, **(II^{det})** or **(III^{det})**, and are different from each other; and

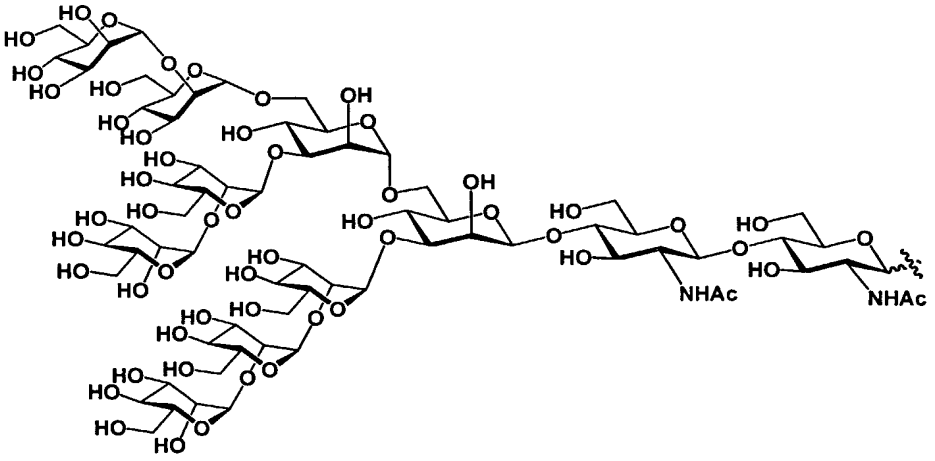
R^{X2} is hydrogen, alkyl, acyl, aryl, heteroaryl, -alkyl(aryl), -alkyl(heteroaryl) or a nitrogen protecting group.

43. The construct of claim 42 having the structure:

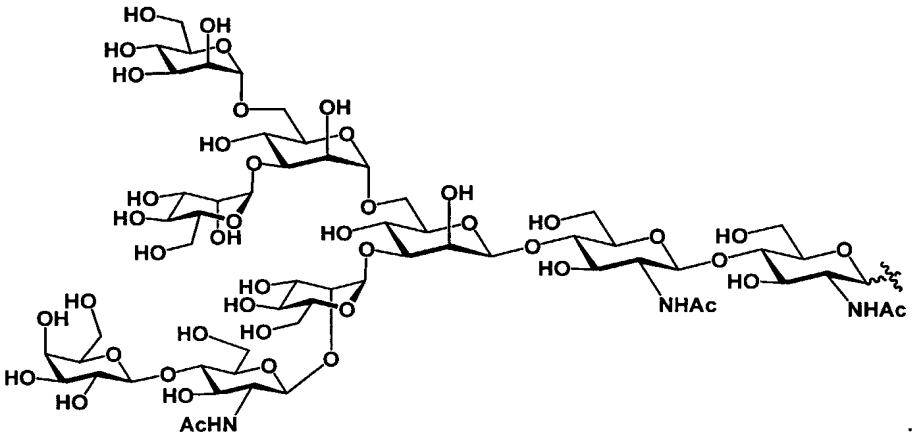


wherein R, R^{X2}, R^{sp}, s1, s2 and s3 and A_1-A_3 are as defined in claim 30; each occurrence of n is independently an integer from 1-10; and each occurrence of R^{aa} is hydrogen, lower alkyl, aryl, heteroaryl, -alkyl(aryl) or –alkyl(heteroaryl).

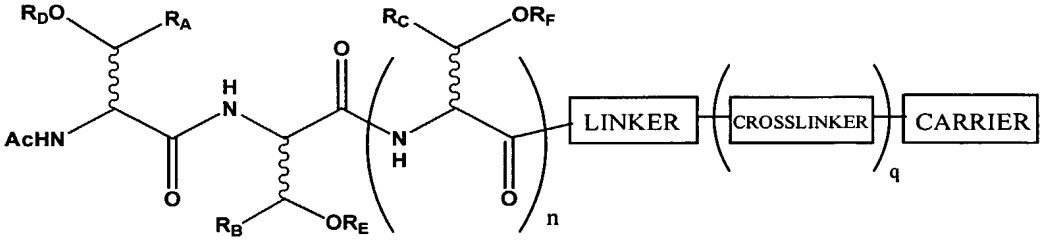
44. The construct of claim 37 or 40, wherein each occurrence of A is independently a carbohydrate domain having one of the following structures:



or



45. The construct of claim 40 having the structure:



wherein the linker is -O-, -NR_G-, -$NR_G(CR_HR_J)_kNR_K$-, $NR_G(CR_HR_J)_kNR_K(C=O)(CR_HR_J)_kS$-, -$(CR_HR_J)_kNR_K$-, -$O(CR_HR_J)_kNR_K$-, an oligoester fragment comprising from 2 to about 20 hydroxy acyl residues, a peptidic fragment comprising from 2 to about 20 amino acyl residues, or a linear or branched chain alkyl or aryl carboxylic ester, wherein each occurrence of k is independently 1-5;

wherein each occurrence of R_G, R_H, R_J or R_K is independently hydrogen, a linear or branched, substituted or unsubstituted, cyclic or acyclic alkyl moiety, or a substituted or unsubstituted aryl moiety;

wherein the crosslinker is a moiety derived from a crosslinking reagent capable of

conjugating the carrier with the linker;

wherein the carrier is a peptide, protein or lipid;

wherein n is 1, 2, 3 or 4;

wherein q is 0 or 1;

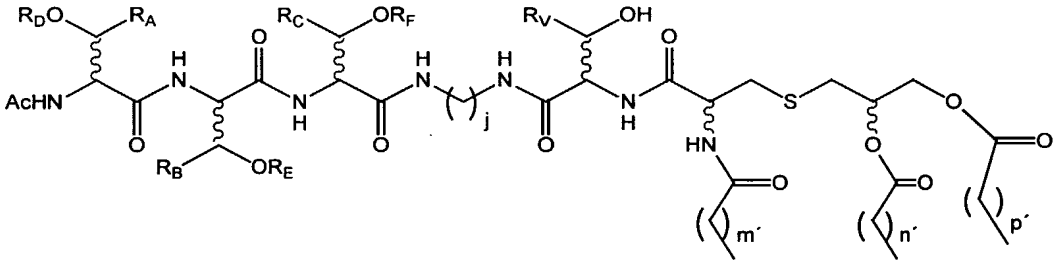
wherein each occurrence of R_A, R_B and R_C is independently hydrogen, substituted or unsubstituted linear or branched chain lower alkyl or substituted or unsubstituted phenyl; and

wherein each occurrence of R_D, R_E and R_F are each independently a carbohydrate domain of formula **(I^{det})**, **(II^{det})** or **(III^{det})**.

46. The construct of claim 37, 40 or 45 wherein the linker is a moiety having the structure $-NH(CH_2)_tNHC(=O)(CH_2)_vS-$ wherein t and v are each integers from 1-6.

47. The construct of claim 46 wherein , t is 3 and v is 1.

48. The construct of claim 40 having the structure:



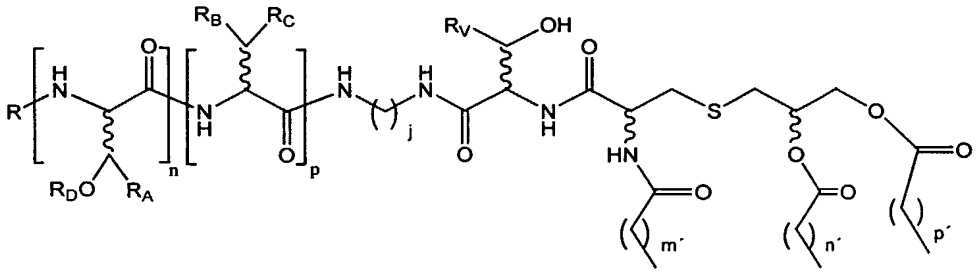
wherein m′, n′ and p′ are integers between about 8 and 20;

j is an integer between 1 and about 8;

R_V, R_A, R_B and R_C are independently hydrogen, substituted or unsubstituted linear or branched chain lower alkyl or substituted or unsubstituted phenyl; and

R_D, R_E and R_F are each independently a carbohydrate domain of formula **(I^{det})**, **(II^{det})** or **(III^{det})**.

49. The construct of claim 40 having the structure:



wherein n and p are each independently an integer from 1-6;

m′, n′ and p′ are independently integers between about 8 and 20;

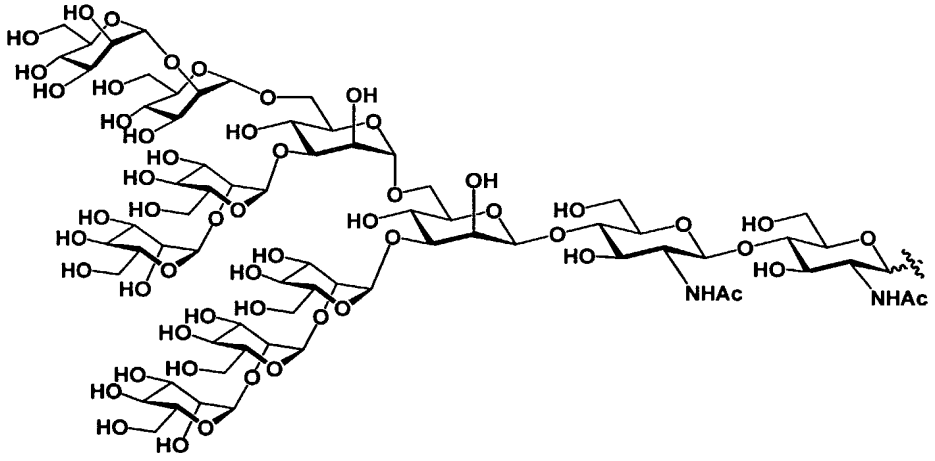
j is an integer between 1 and about 8;

R is a nitrogen protecting group;

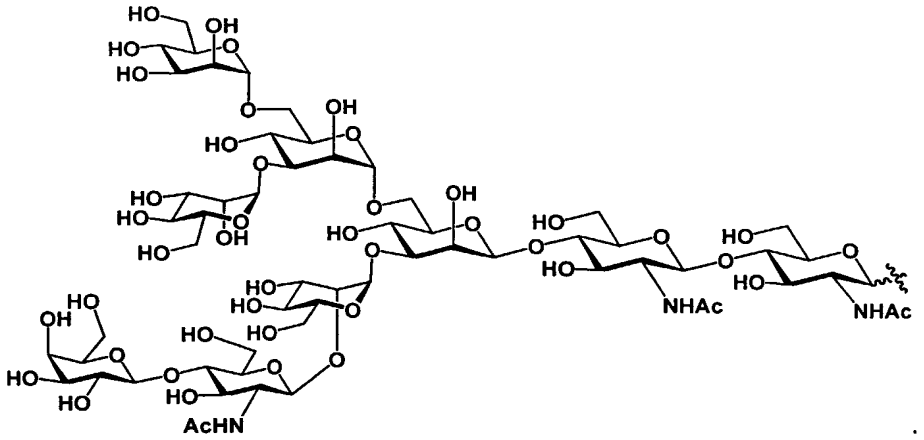
R_V, and R_A, R_B, R_C, R_E and R_F, for each occurrence, are independently hydrogen, substituted or unsubstituted linear or branched lower alkyl or substituted or unsubstituted phenyl;

each occurrence of R_D is independently a carbohydrate domain of formula **(I^{det})**, **(II^{det})** or **(III^{det})**.

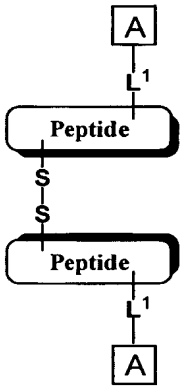
50. The construct of claim 48 or 49 wherein each occurrence of R_D, R_E and R_F is independently a carbohydrate domain having one of the following structures:



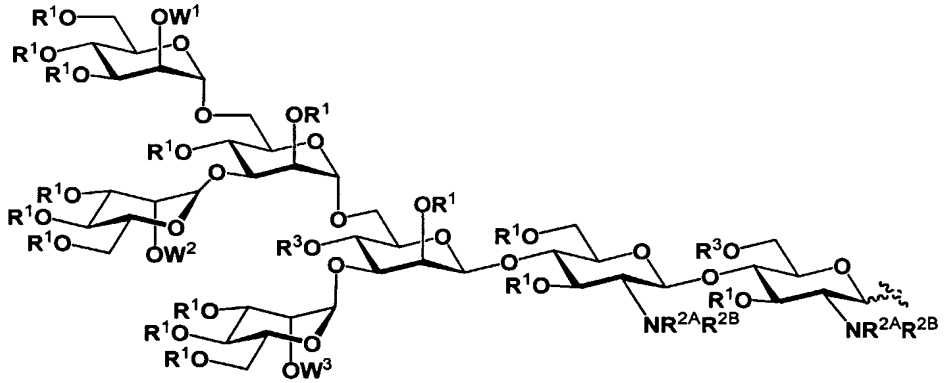
or



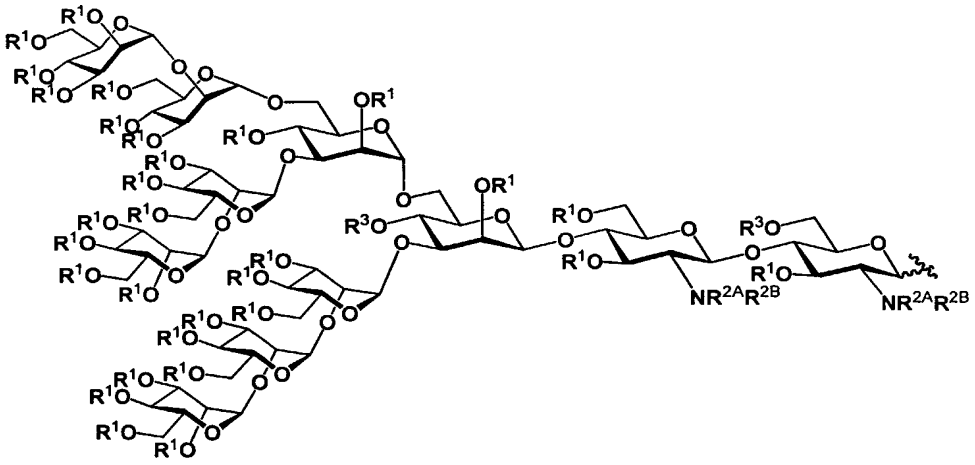
51. A dimeric glycopeptide having the structure:



wherein each peptide may be the same or different; and each occurrence of A is independently a carbohydrate domain having the structure:

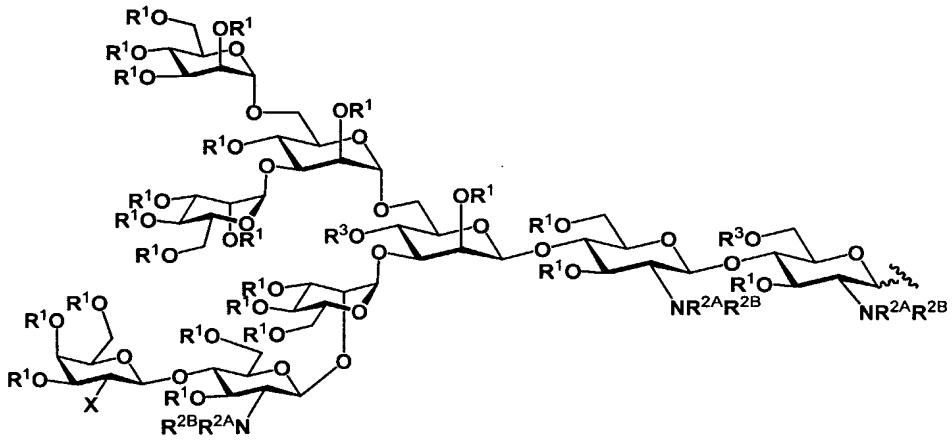


(I^{det})



(II^{det})

or

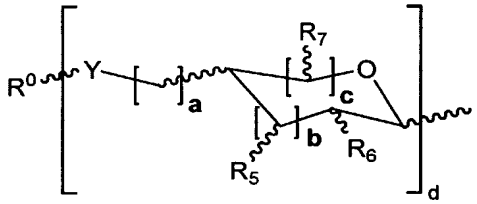


(III^{det})

wherein each occurrence of R^1 is independently hydrogen or an oxygen protecting group;

each occurrence of R^{2A} and R^{2B} is independently hydrogen or a nitrogen protecting group;

each occurrence of R^3 is independently hydrogen, a protecting group or a carbohydrate domain comprising a saccharide moiety having the structure:



wherein Y is NH or O; wherein a, b and c are each independently 0, 1 or 2; d is an integer from 1-3; with the proviso that the d bracketed structure represents a furanose or pyranose moiety and the sum of b and c is 1 or 2; wherein R^0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein each occurrence of R^5, R^6 and R^7 is independently hydrogen, OH, OR^i, $NR^{ii}R^{iii}$, $NHCOR^i$, F, CH_2OH, CH_2OR^i, or a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of R^i, R^{ii} and R^{iii} is independently hydrogen, a protecting group, a sialic acid moiety, CHO, $COOR^{iv}$, or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group, or R^{ii} and R^{iii}, taken together with the nitrogen atom to which they are attached, form a substituted or unsubstituted heterocyclic or heteroaryl moiety; and wherein each occurrence of R^{iv} is independently H, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group;

W^1, W^2 and W^3 are independently optionally substituted mannose, galactose or lactosamine moieties.

52. The glycopeptide of claim 51, wherein each occurrence of L^1 is independently $-O(CHR^{aa})_n-$ or $-NHC(=O)(CHR^{aa})_n-$ wherein each occurrence of n is independently an integer from 1-10; and each occurrence of R^{aa} is hydrogen, lower alkyl, aryl, heteroaryl, -alkyl(aryl) or –alkyl(heteroaryl).

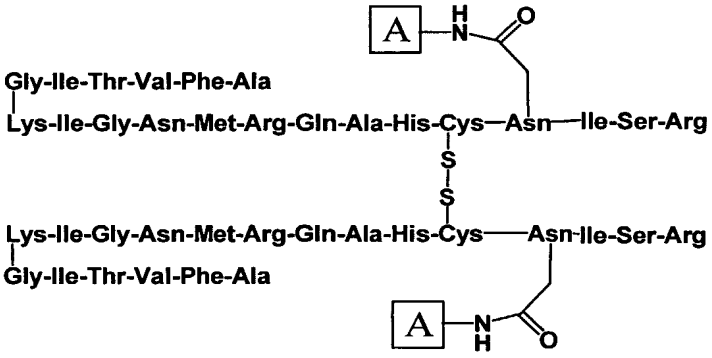
53. The glycopeptide of claim 52, wherein each occurrence of L^1 is an aspartyl side chain.

54. The glycopeptide of claim 52, wherein the peptide has a structure that is either identical or closely related to that of gp120 near an N-glycosylation site.

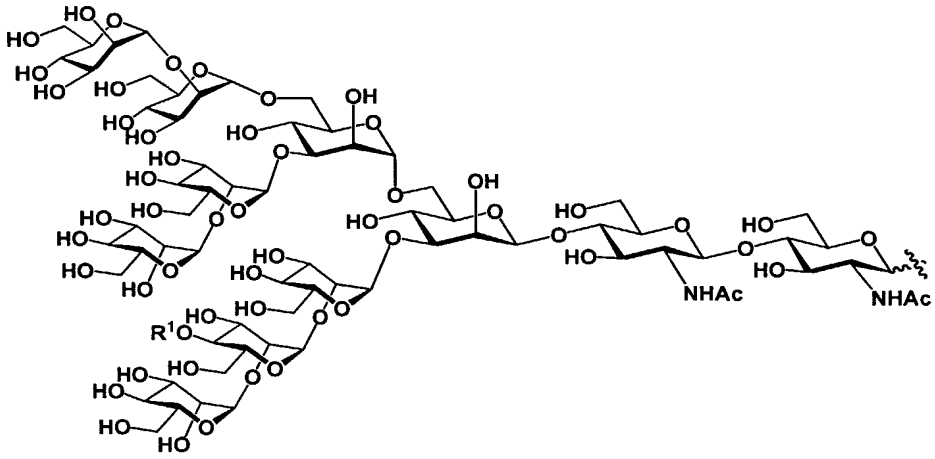
55. The glycopeptide of claim 54, wherein the peptide comprises the amino acid sequence: Cys-Asn-Ile-Ser-Arg, wherein any one or more of the amino acid residues may bear one or more protecting groups.

56. The glycopeptide of claim 54, wherein the peptide comprises the amino acid sequence: Ala-Phe-Val-Thr-Ile-Gly-Lys-Ile-Gly-Asn-Met-Arg-Gln-Ala-His-Cys-Asn-Ile-Ser-Arg, wherein any one or more of the amino acid residues may bear one or more protecting groups.

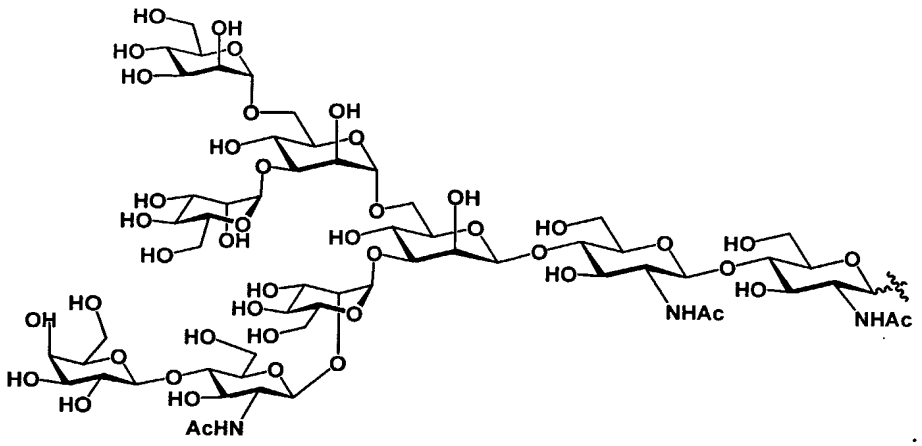
57. The glycopeptide of claim 52 having the structure:



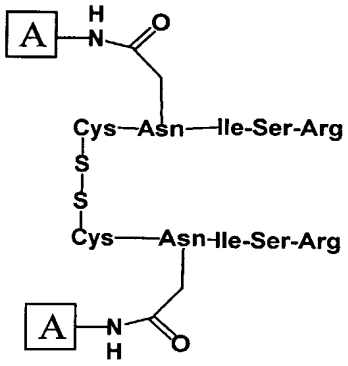
wherein each occurrence of A is independently a carbohydrate domain having one of the structures:



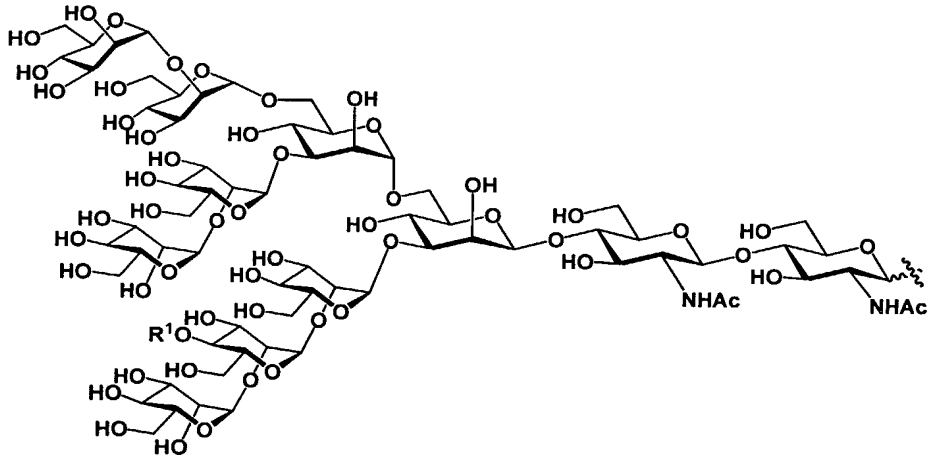
or



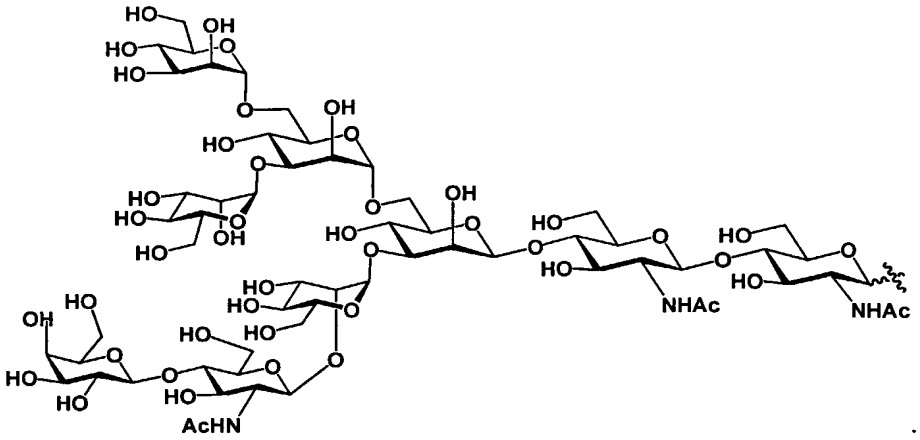
58. The glycopeptide of claim 52 having the structure:



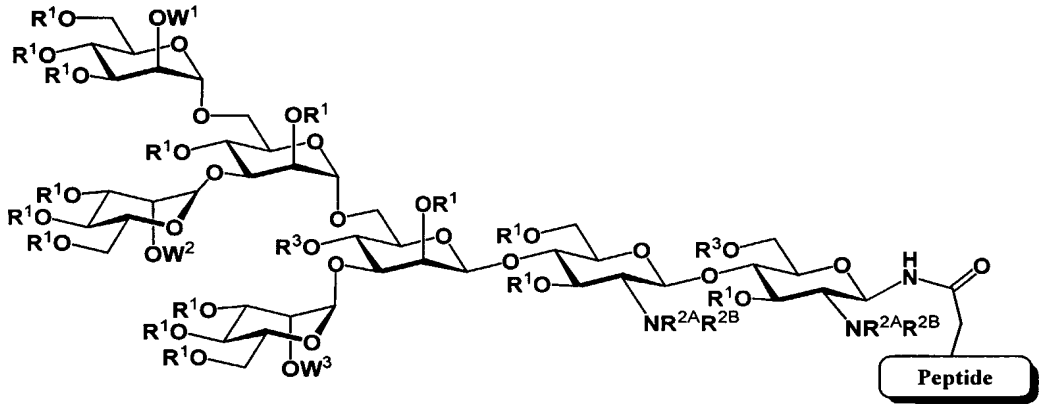
wherein each occurrence of A is independently a carbohydrate domain having one of the structures:



or



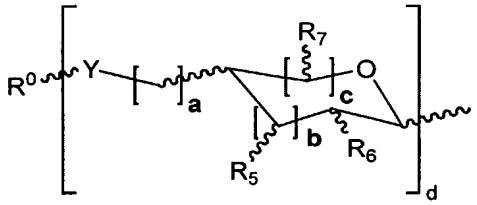
59. A method for preparing an isolated compound having the structure:



wherein each occurrence of R^1 is independently hydrogen or an oxygen protecting group;

each occurrence of R^{2A} and R^{2B} is independently hydrogen or a nitrogen protecting group;

each occurrence of R^3 is independently hydrogen, a protecting group or a carbohydrate domain comprising a saccharide moiety having the structure:



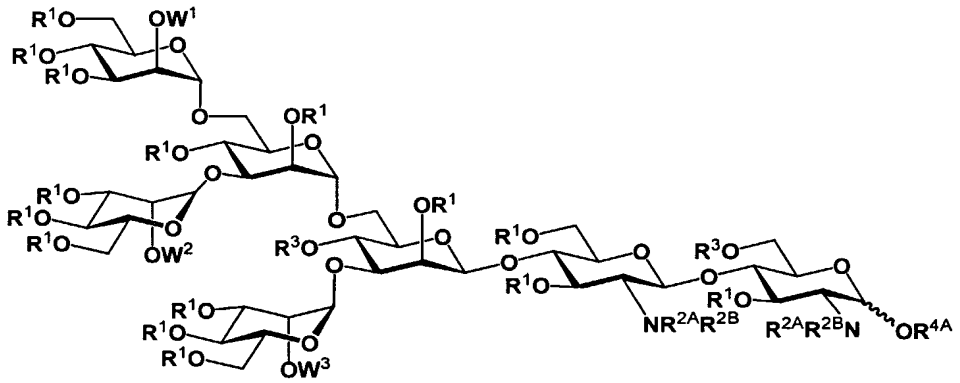
wherein Y is NH or O; wherein a, b and c are each independently 0, 1 or 2; d is an integer from 1-3; with the proviso that the d bracketed structure represents a furanose or pyranose moiety and the sum of b and c is 1 or 2; wherein R^0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein

each occurrence of R^5, R^6 and R^7 is independently hydrogen, OH, OR^i, $NR^{ii}R^{iii}$, $NHCOR^i$, F, CH_2OH, CH_2OR^i, or a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of R^i, R^{ii} and R^{iii} is independently hydrogen, a protecting group, a sialic acid moiety, CHO, $COOR^{iv}$, or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group, or R^{ii} and R^{iii}, taken together with the nitrogen atom to which they are attached, form a substituted or unsubstituted heterocyclic or heteroaryl moiety; and wherein each occurrence of R^{iv} is independently H, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group;

W^1, W^2 and W^3 are independently optionally substituted mannose, galactose or lactosamine moieties;

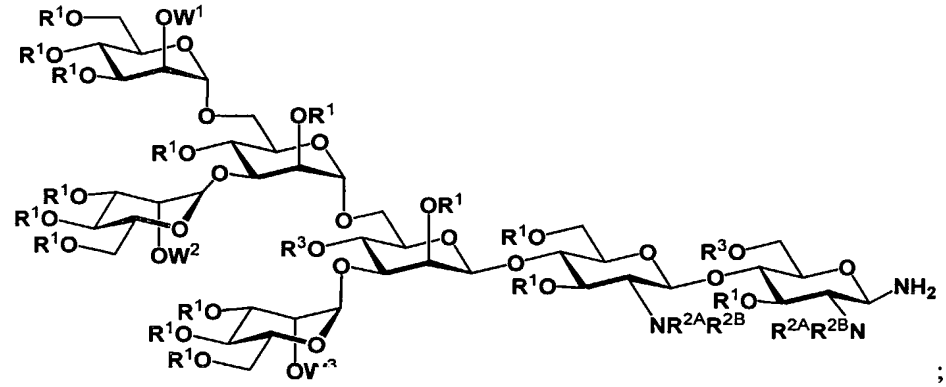
said method comprising steps of:

(a) providing an α-O-protected carbohydrate construct having the structure:



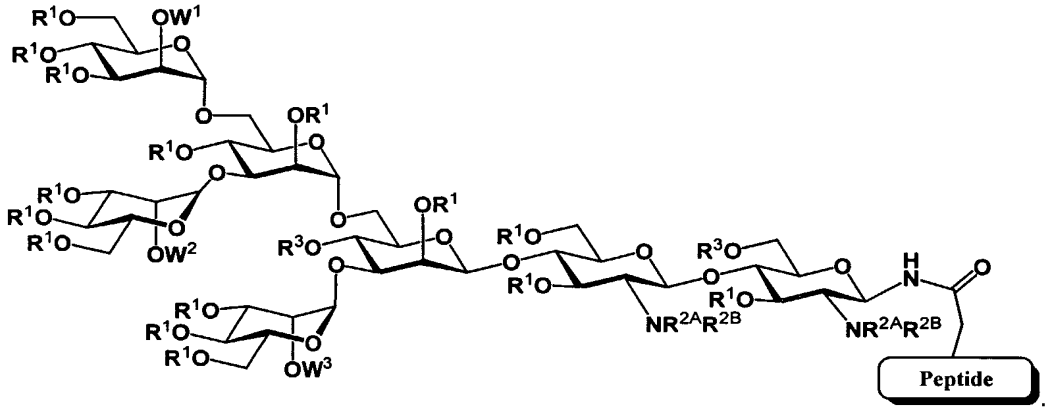
wherein R^{4A} is hydrogen or a suitable oxygen protecting group;

(b) reacting the construct of step (a) under suitable conditions to form a β-amino carbohydrate construct having the structure:



;

(c) reacting said β-amino carbohydrate construct under suitable conditions with a peptide whose structure is either identical or closely related to that of gp120 near an N-glycosylation site and which comprises a $-CH_2CO_2H$ moiety, to form a glycopeptide having the structure:



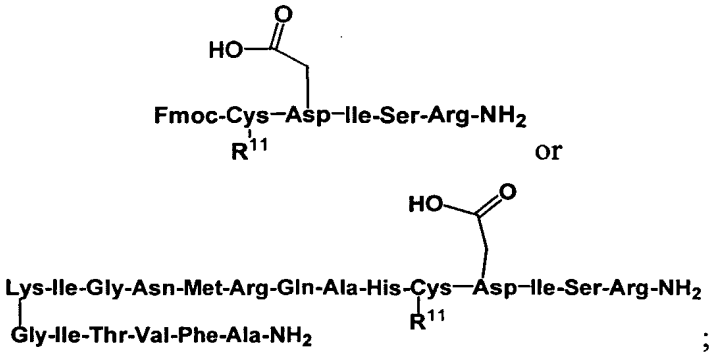
60. The method of claim 59, wherein in the step of reacting the carbohydrate construct of step (a) under suitable conditions to form the β-amino carbohydrate construct, Kochetkov amination conditions are used.

61. The method of claim 60, wherein NH_4HCO_3/H_2O is used.

62. The method of claim 59, wherein, in the β-amino carbohydrate construct of step (b), each occurrence of R^1 and R^3 is hydrogen and each occurrence of $-NR^{2A}R^{2B}$ is –NHAc.

63. The method of claim 59, wherein, in the step of reacting the β-amino carbohydrate construct under suitable conditions with a peptide whose structure is either identical or closely related to that of gp120 near an N-glycosylation site, the reaction conditions comprise HATU and Hünig's base is a suitable solvent.

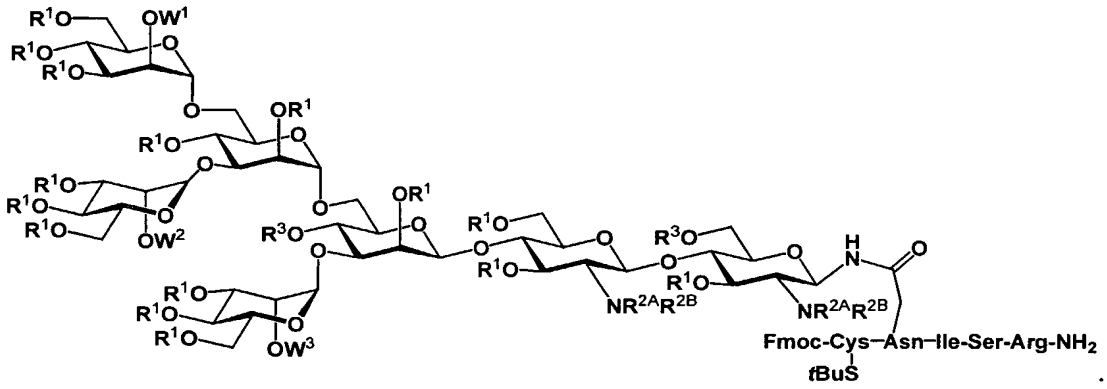
64. The method of claim 63, wherein the peptide has the following structure:



;

wherein R^{11} is hydrogen or –S*t*Bu.

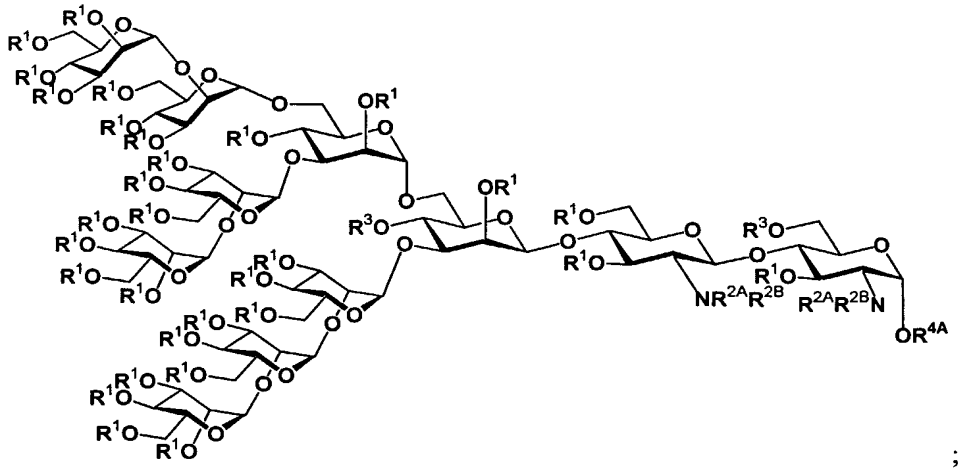
65. The method of claim 59, wherein the glycopeptide of step (c) has the structure:



.

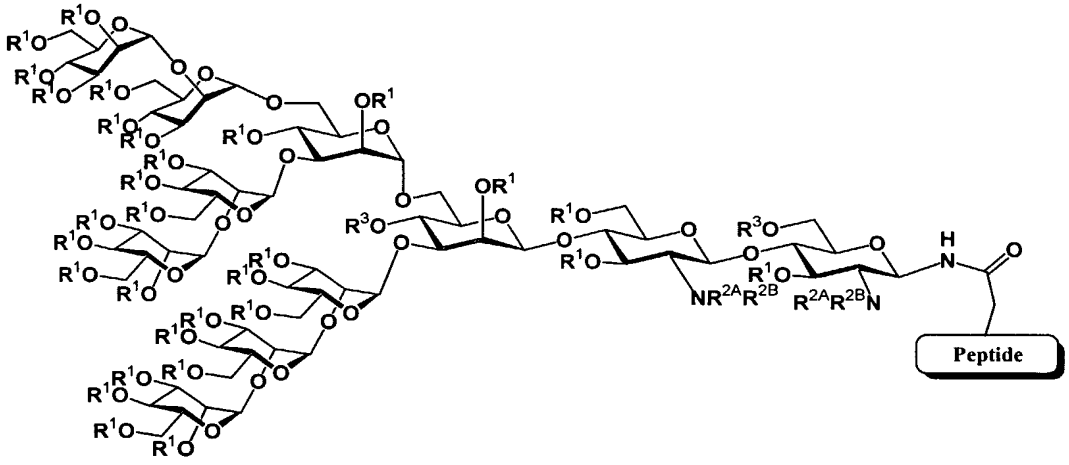
66. The method of claim 59, wherein, in the β-amino carbohydrate construct formed in step (b), each occurrence of R^1 is hydrogen, each occurrence of $-NR^{2A}R^{2B}$ is –NHAc.

67. The method of claim 59, wherein the α-O-protected carbohydrate construct of step (a) has the structure:



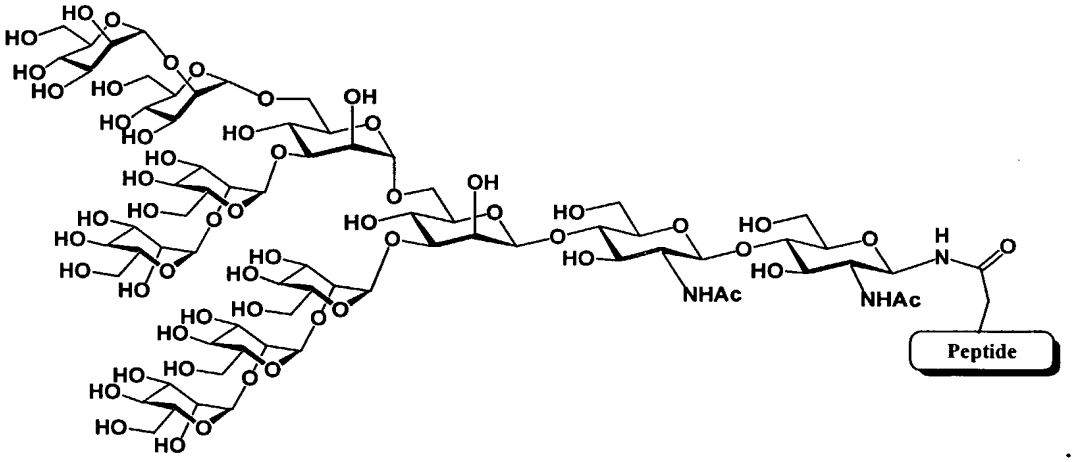
;

and the glycopeptide formed in step (c) has the structure:

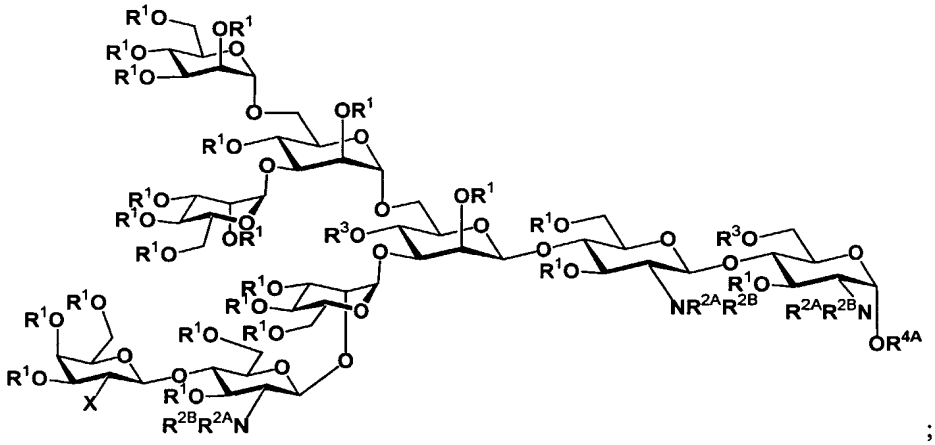


68. The method of claim 59, wherein the α-O-protected carbohydrate construct of step (a) has the structure:

and the glycopeptide formed in step (c) has the structure:

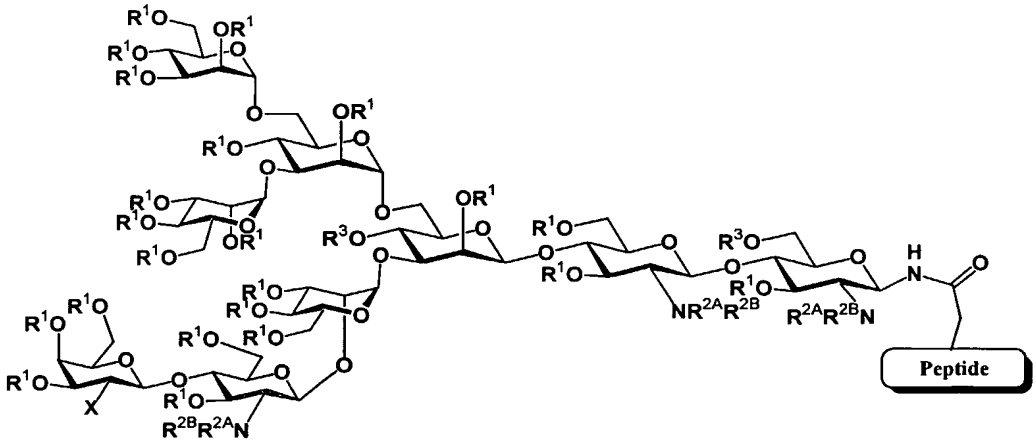


69. The method of claim 59, wherein the α-O-protected carbohydrate construct of step (a) has the structure:

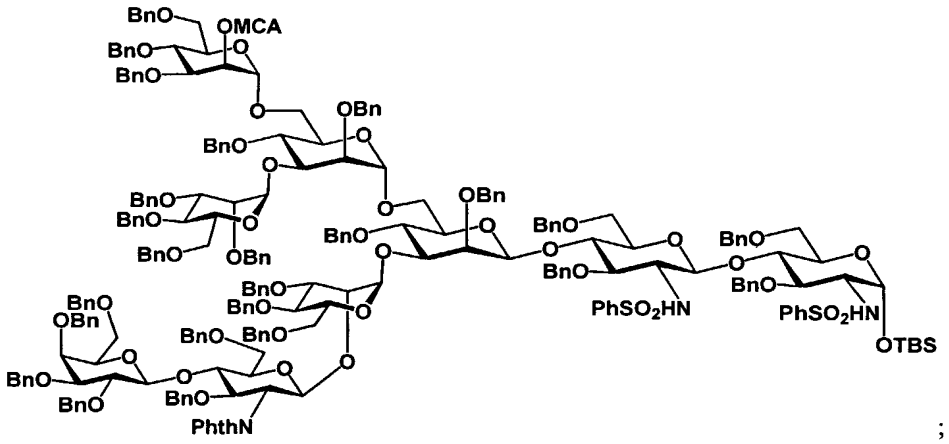


;

and the glycopeptide formed in step (c) has the structure:



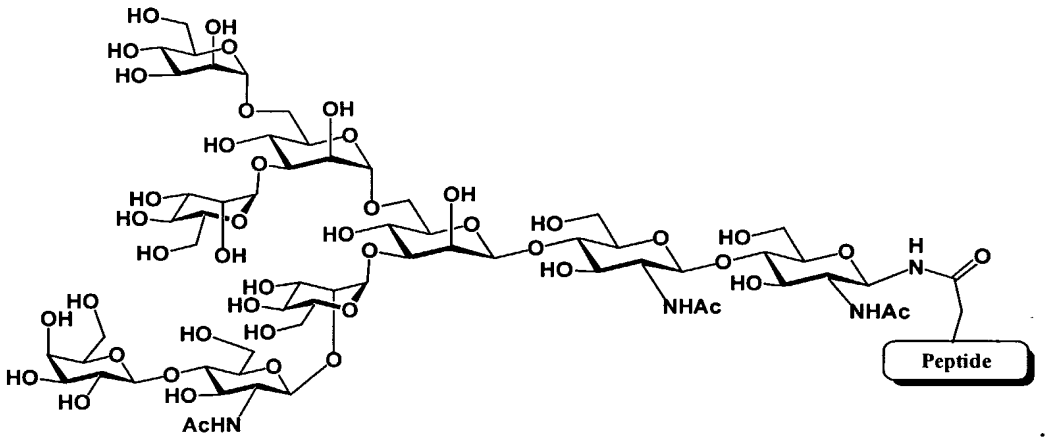
70. The method of claim 59, wherein the α-O-protected carbohydrate construct of step (a) has the structure:



;

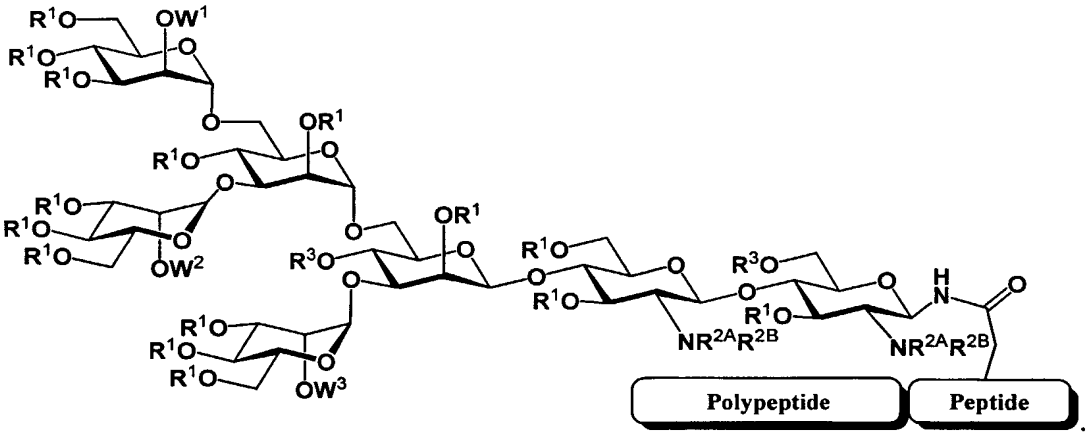
wherein MCA represent monochloroacetate

and the glycopeptide formed in step (c) has the structure:



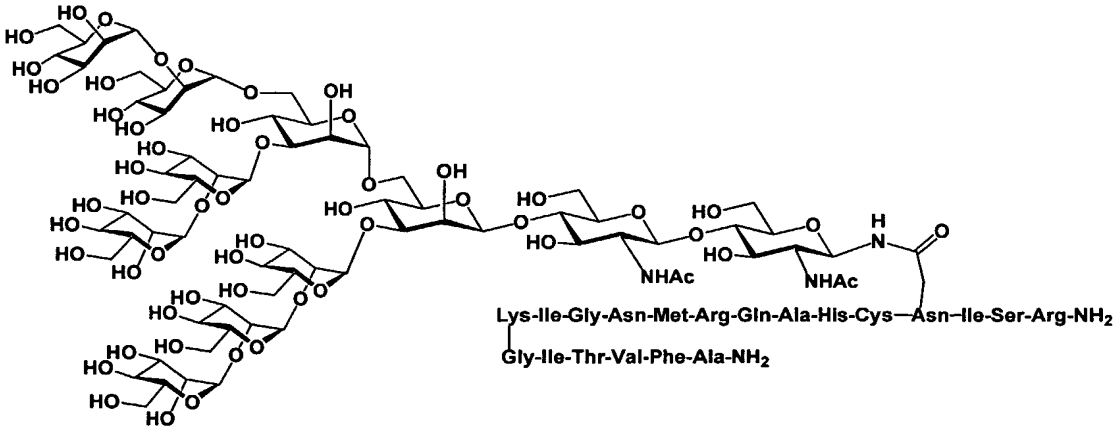
.

71. The method of claim 59, further comprising a step of subjecting the glycopeptide formed in step (c) to Native Chemical Ligation conditions in the presence of a suitable polypeptide to form a glycopolypeptide having the structure:



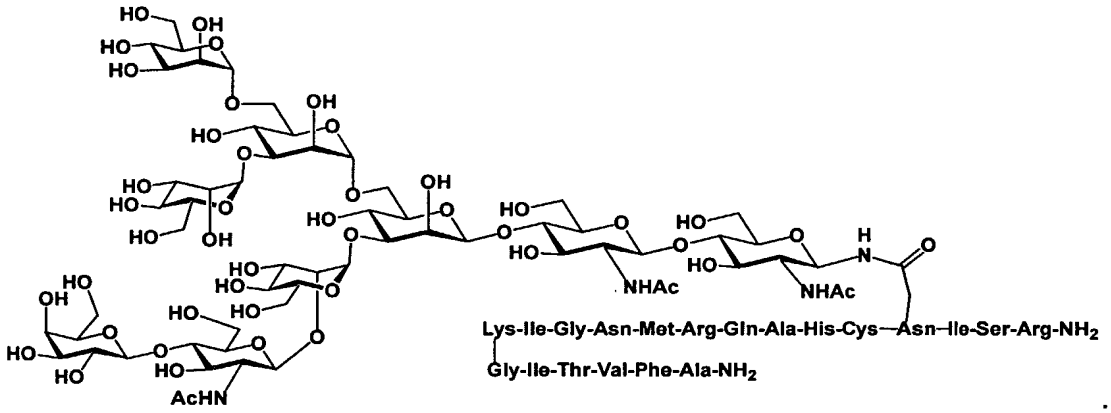
72. The method of claim 71, wherein the polypeptide comprises the amino acid sequence: Ala-Phe-Val-Thr-Ile-Gly-Lys-Ile-Gly-Asn-Met-Arg-Gln-Ala-His-Cys-Asn-Ile-Ser-Arg wherein any one or more of the amino acid residues may bear one or more protecting groups or a moiety suitable for Native Chemical Ligation.

73. The method of claim 71, wherein the polypeptide has the structure: Ala-Phe-Val-Thr-Ile-Gly-Lys-Ile-Gly-Asn-Met-Arg-Gln-Ala-His –SR; where R is a functional group suitable for effecting chemical ligation; and the resulting glycopeptide has the structure:

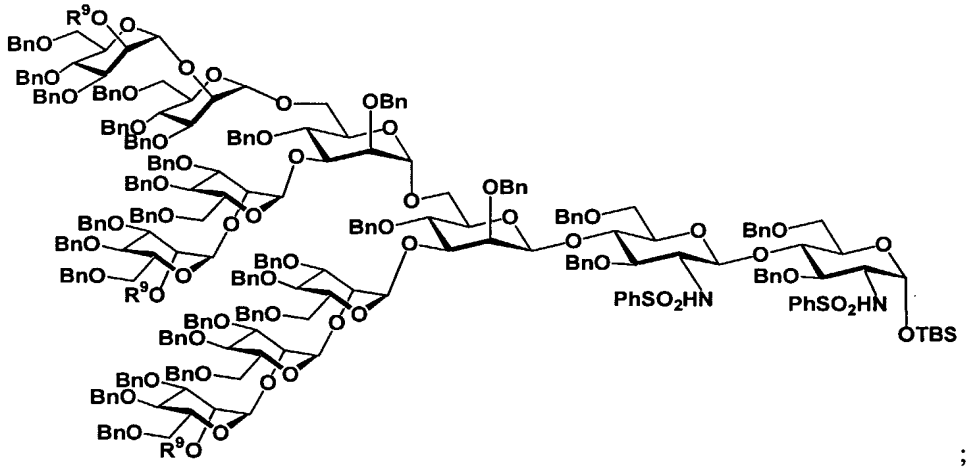


74. The method of claim 71, wherein the polypeptide has the structure:

Ala-Phe-Val-Thr-Ile-Gly-Lys-Ile-Gly-Asn-Met-Arg-Gln-Ala-His –SR; where R is a functional group suitable for effecting chemical ligation; and the resulting glycopeptide has the structure:



75. A method for preparing an α-O-protected carbohydrate construct having the structure:

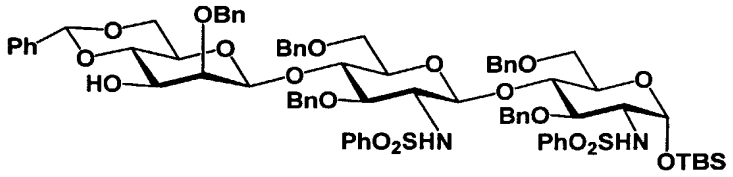


;

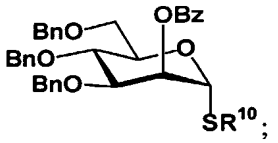
wherein each occurrence of R^9 is independently Bz or Ac;

said method comprising steps of:

(a) coupling a trisaccharide having the structure:

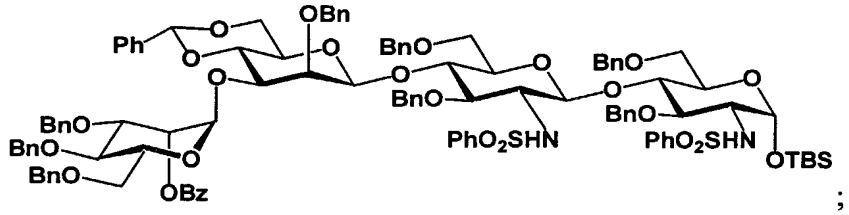


with a monosaccharide having the structure:



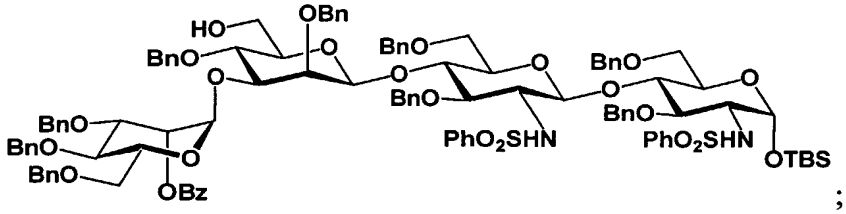
wherein R^{10} is lower alkyl or aryl;

in the presence of an activating agent under suitable conditions to form a protected tetrasaccharide having the structure:



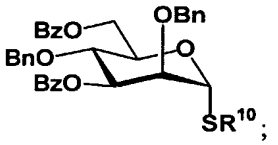
;

(b) partially deprotecting the protected tetrasaccharide formed in step (a) under suitable conditions to form a partially deprotected tetrasaccharide having the structure:



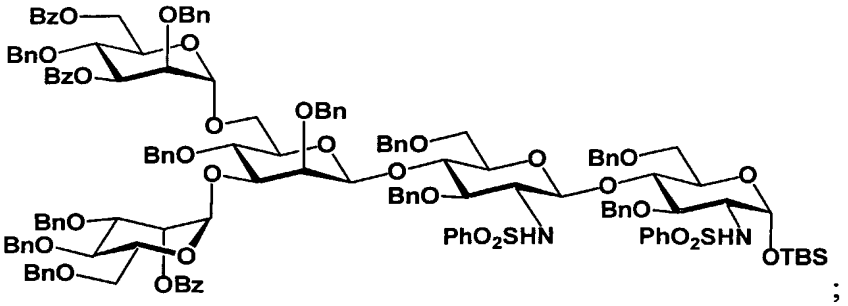
;

(c) coupling the partially deprotected tetrasaccharide formed in step (b) with a monosaccharide having the structure:



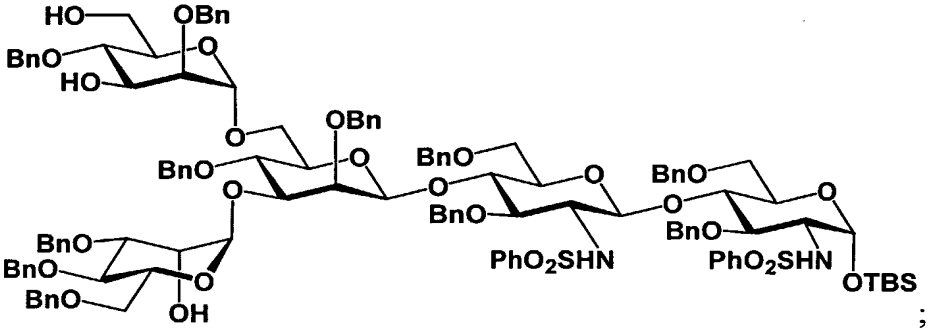
wherein R^{10} is lower alkyl or aryl;

in the presence of an activating agent under suitable conditions to form a protected pentasaccharide having the structure:



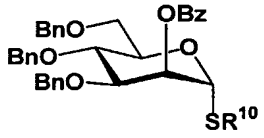
;

(d) partially deprotecting the pentasaccharide formed in step (c) under suitable conditions to form a partially deprotected pentasaccharide having the structure:



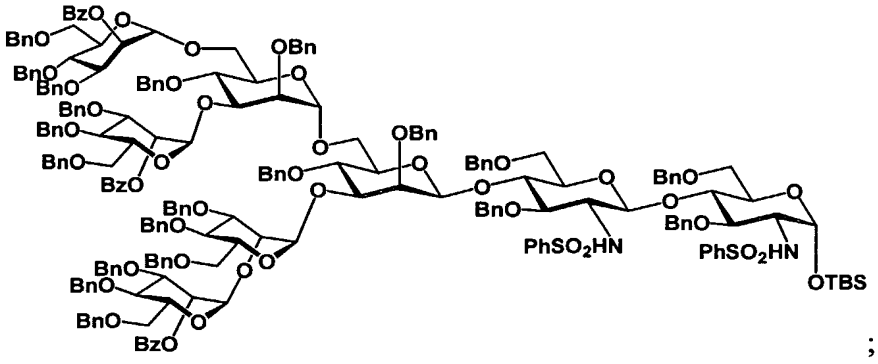
;

(e) coupling the partially deprotected pentasaccharide formed in step (d) with a monosaccharide having the structure:



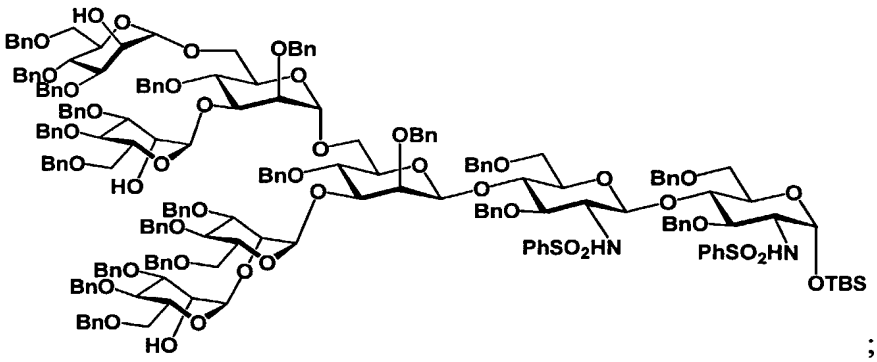
wherein R^{10} is lower alkyl or aryl;

in the presence of an activating agent under suitable conditions to form an octasaccharide having the structure:



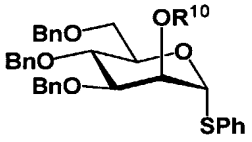
; and

(f) partially deprotecting the octasaccharide formed in step (e) under suitable conditions to form a partially deprotected octasaccharide having the structure:



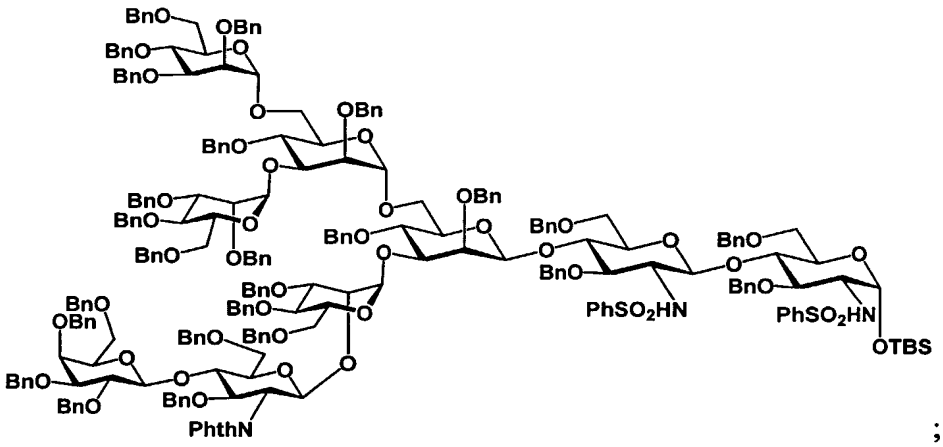
; and

(g) coupling the partially deprotected octasaccharide formed in step (f) with a monosaccharide having the structure:



in the presence of an activating agent under suitable conditions to the α-O-protected carbohydrate construct.

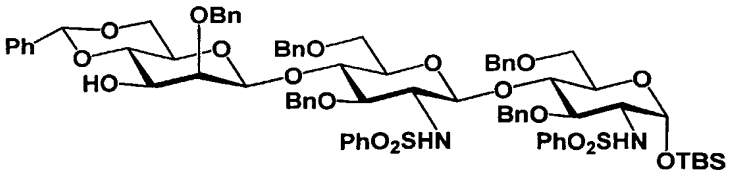
76. A method for preparing an α-O-protected carbohydrate construct having the structure:



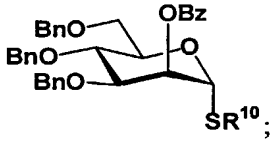
;

said method comprising steps of:

(a) coupling a trisaccharide having the structure:

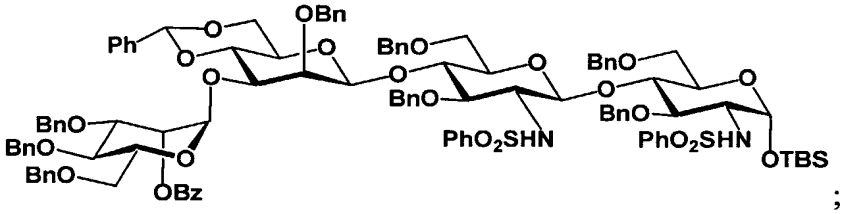


with a monosaccharide having the structure:



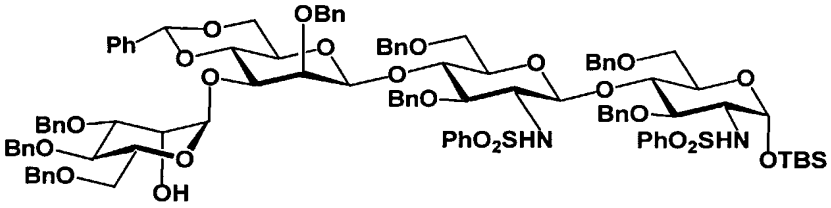
wherein R^{10} is lower alkyl or aryl;

in the presence of an activating agent under suitable conditions to form a protected tetrasaccharide having the structure:

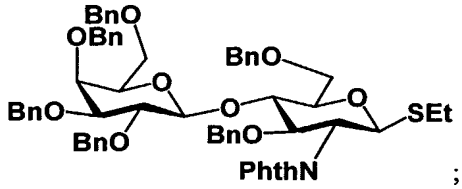


;

(b) partially deprotecting the protected tetrasaccharide formed in step (a) under suitable conditions to form a partially deprotected tetrasaccharide having the structure:

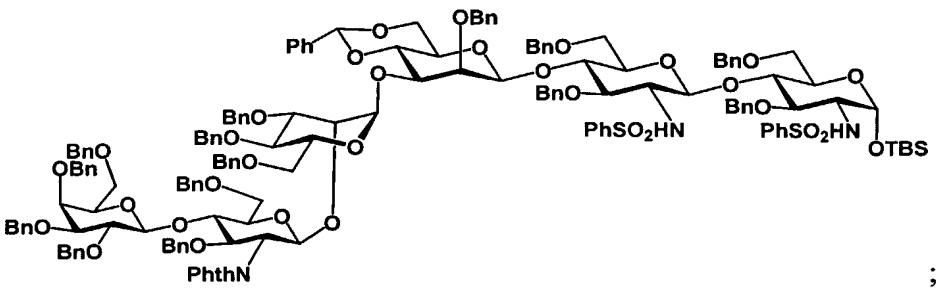


(c) coupling the partially deprotected tetrasaccharide formed in step (b) with an ethylthioglycoside having the structure:



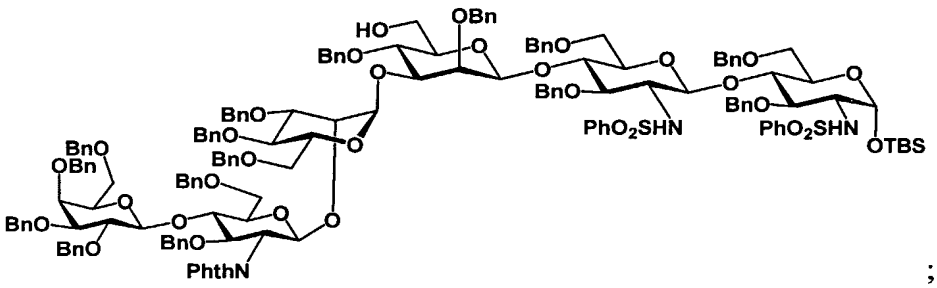
;

under suitable conditions to form a protected hexasaccharide having the structure:



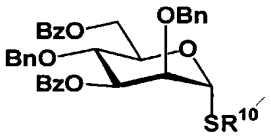
;

(d) partially deprotecting the hexasaccharide formed in step (c) under suitable conditions to form a partially deprotected hexasaccharide having the structure:



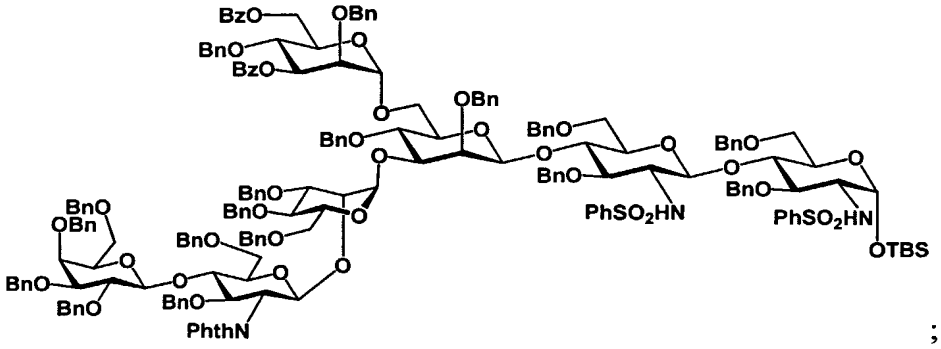
;

(e) coupling the partially deprotected hexasaccharide formed in step (d) with a monosaccharide having the structure:



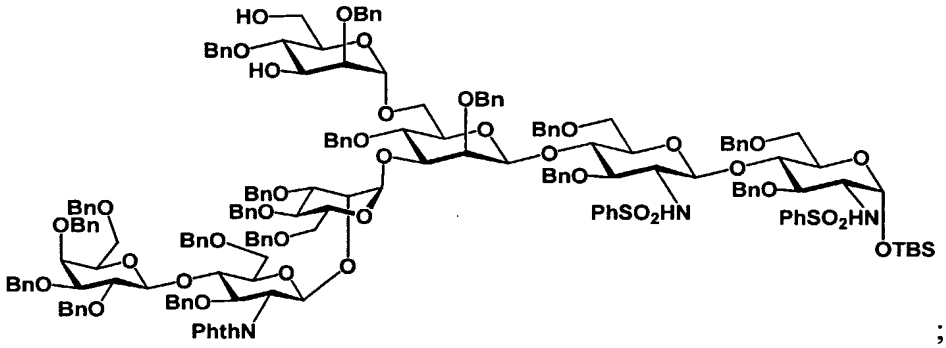
wherein R^{10} is lower alkyl or aryl;

in the presence of an activating agent under suitable conditions to form an heptasaccharide having the structure:



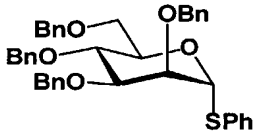
; and

(f) partially deprotecting the heptasaccharide formed in step (e) under suitable conditions to form a partially deprotected heptasaccharide having the structure:



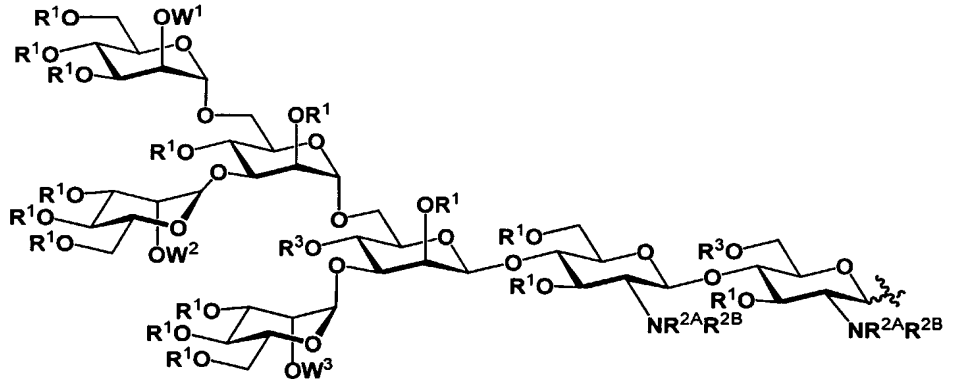
; and

(g) coupling the partially deprotected heptasaccharide formed in step (f) with a monosaccharide having the structure:



in the presence of an activating agent under suitable conditions to the α-O-protected carbohydrate construct.

77. An antibody or antibody fragment which is specific to any one or more of the carbohydrate antigens present on a multi-antigenic glycoconjugate comprising one or more carbohydrate domains having the structure:

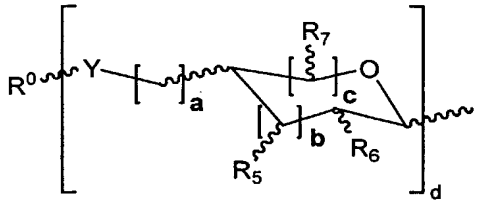


(I)

wherein each occurrence of R^1 is independently hydrogen or an oxygen protecting group;

each occurrence of R^{2A} and R^{2B} is independently hydrogen or a nitrogen protecting group;

each occurrence of R^3 is independently hydrogen, a protecting group or a carbohydrate domain comprising a saccharide moiety having the structure:



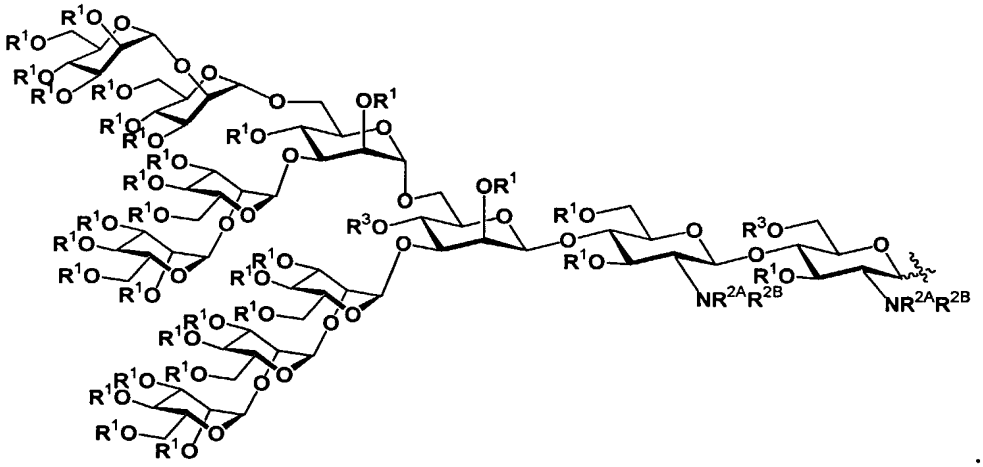
wherein Y is NH or O; wherein a, b and c are each independently 0, 1 or 2; d is an integer from 1-3; with the proviso that the d bracketed structure represents a furanose or pyranose moiety and the sum of b and c is 1 or 2; wherein R^0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein each occurrence of R^5, R^6 and R^7 is independently hydrogen, OH, OR^i, $NR^{ii}R^{iii}$, $NHCOR^i$, F, CH_2OH, CH_2OR^i, or a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of R^i, R^{ii} and R^{iii} is independently hydrogen, a protecting group, a sialic acid moiety, CHO, $COOR^{iv}$, or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group, or R^{ii} and R^{iii}, taken together with the nitrogen atom to which they are attached, form a substituted or unsubstituted heterocyclic or heteroaryl moiety; and wherein each occurrence of R^{iv} is independently H, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group;

W^1, W^2 and W^3 are independently optionally substituted mannose, galactose or lactosamine moieties;

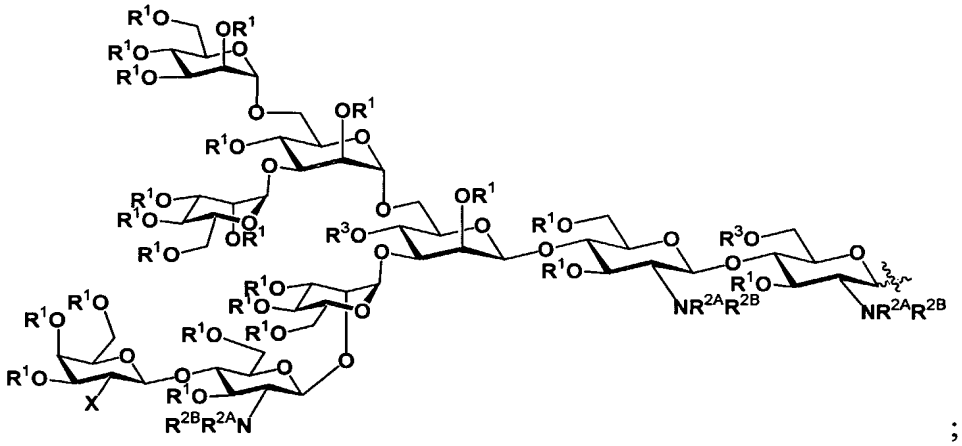
wherein each carbohydrate domain is independently covalently bound to a linker system, said linker system being a peptide or non-peptide nature; and wherein the linker system may be cyclic or acyclic; and

wherein said antibody is a purified polyclonal antibody or a monoclonal antibody.

78. The antibody or antibody fragment of claim 77, wherein, in the antigen, a carbohydrate domain has the structure:



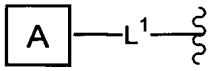
79. The antibody or antibody fragment of claim 77, wherein a antigen has the structure:



;

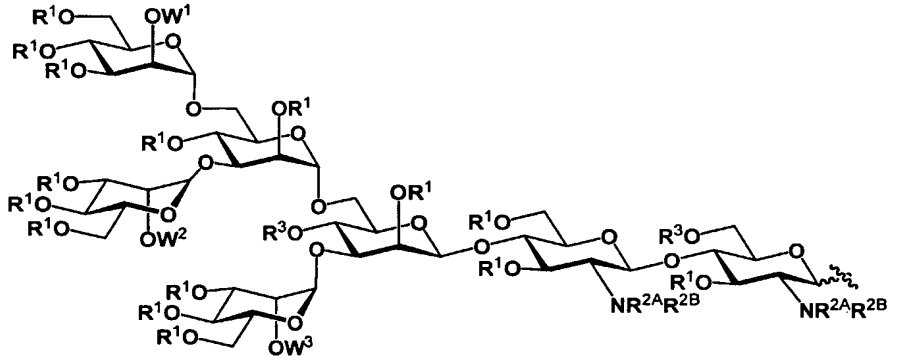
wherein X is OR^1 or $NR^{2A}R^{2B}$.

80. The antibody or antibody fragment of claim 77, wherein the antigen is a multi-antigenic construct comprising a cyclic or acyclic peptidic or non-peptidic backbone made up of two or more structural units, wherein one or more of said structural units is/are independently substituted with a glycosidic moiety having the structure:

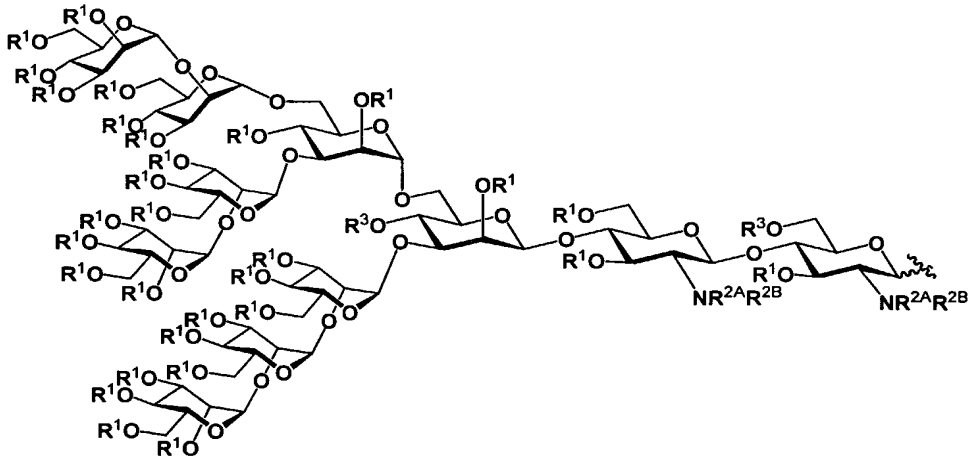


wherein each occurrence of L^1 is independently a substituted or unsubstituted, linear or branched, cyclic or acyclic, saturated or unsaturated aliphatic or heteroaliphatic moiety; and

each occurrence of A is independently a carbohydrate domain of formula:

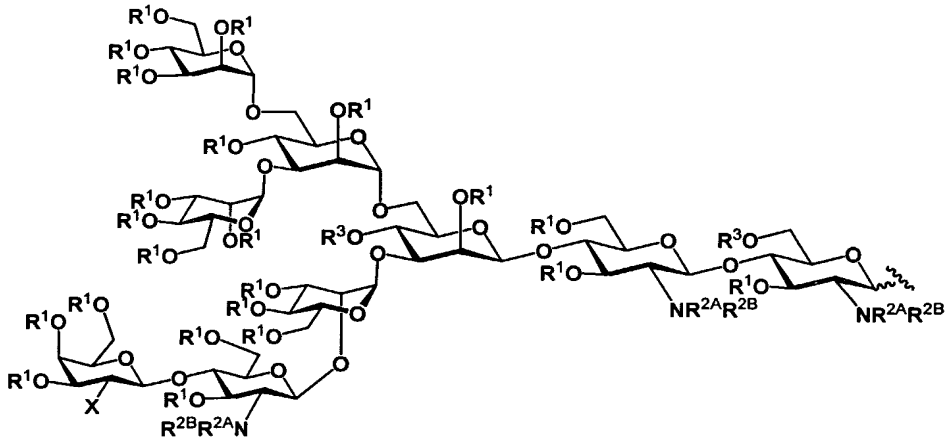


(I^{det})



(II^{det})

or

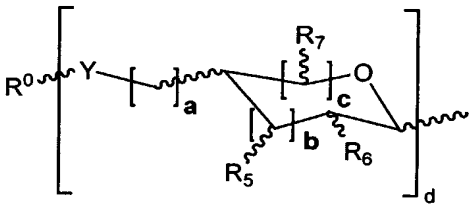


(III^{det})

wherein each occurrence of R^1 is independently hydrogen or an oxygen protecting group;

each occurrence of R^{2A} and R^{2B} is independently hydrogen or a nitrogen protecting group;

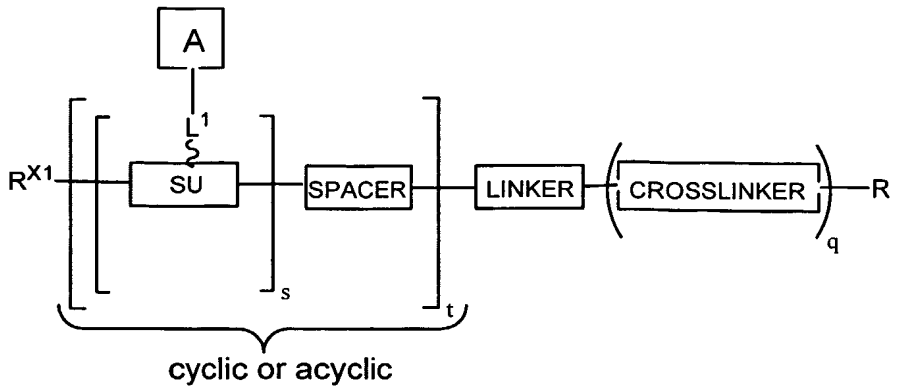
each occurrence of R^3 is independently hydrogen, a protecting group or a carbohydrate domain comprising a saccharide moiety having the structure:



wherein Y is NH or O; wherein a, b and c are each independently 0, 1 or 2; d is an integer from 1-3; with the proviso that the d bracketed structure represents a furanose or pyranose moiety and the sum of b and c is 1 or 2; wherein R^0 is hydrogen, a linear or branched chain alkyl, acyl, arylalkyl or aryl group; wherein each occurrence of R^5, R^6 and R^7 is independently hydrogen, OH, OR^i, $NR^{ii}R^{iii}$, $NHCOR^i$, F, CH_2OH, CH_2OR^i, or a substituted or unsubstituted linear or branched chain alkyl, (mono-, di- or tri)hydroxyalkyl, (mono-, di- or tri)acyloxyalkyl, arylalkyl or aryl group; wherein each occurrence of R^i, R^{ii} and R^{iii} is independently hydrogen, a protecting group, a sialic acid moiety, CHO, $COOR^{iv}$, or a substituted or unsubstituted linear or branched chain alkyl, acyl, arylalkyl or aryl group, or R^{ii} and R^{iii}, taken together with the nitrogen atom to which they are attached, form a substituted or unsubstituted heterocyclic or heteroaryl moiety; and wherein each occurrence of R^{iv} is independently H, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group; and

W^1, W^2 and W^3 are independently optionally substituted mannose, galactose or lactosamine moieties.

81. The antibody or antibody fragment of claim 80, wherein the antigen has the structure:



wherein q is 0 or 1;

each occurrence of s is independently an integer from 2-20;

t is an integer from 1-6;

R^{X1} is hydrogen, alkyl, acyl, aryl, heteroaryl, -alkyl(aryl), -alkyl(heteroaryl) or a nitrogen protecting group; or R^{X1} is covalently bound to a substituent on the last occurrence of the spacer, thereby forming a cyclic backbone;

R is hydrogen or an immunogenic carrier;

each occurrence of the structural unit SU is independently a substituted or unsubstituted aliphatic, heteroaliphatic, aryl, heteroaryl or peptidic moiety;

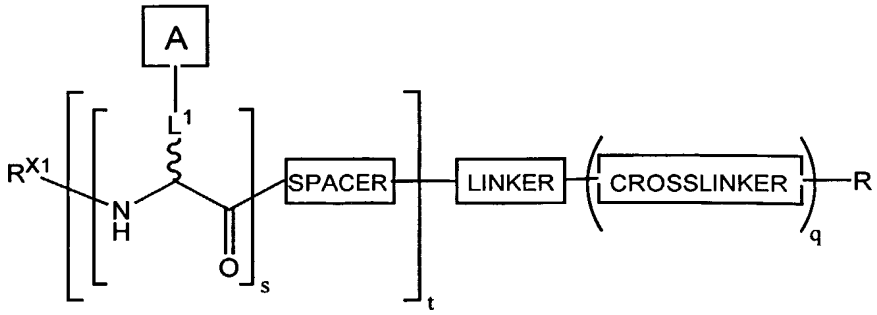
each occurrence of the spacer is independently a substituted or unsubstituted aliphatic, heteroaliphatic, aryl, heteroaryl or peptidic moiety;

the linker is either a free carboxylic acid, –O-, (carboxamido)alkyl carboxamide, MBS, primary carboxamide, mono- or dialkyl carboxamide, mono- or diarylcarboxamide, linear or branched chain (carboxy)alkyl carboxamide, linear or branched chain (alkoxycarbonyl)alkyl-carboxamide, linear or branched chain (carboxy)arylalkylcarboxamide, linear or branched chain (alkoxycarbonyl)alkylcarboxamide, an oligoester fragment comprising from 2 to about 20 hydroxy acyl residues, a peptidic fragment comprising from 2 to about 20 amino acyl residues, or a linear or branched chain alkyl or aryl carboxylic ester;

each occurrence of L^1 is independently a substituted or unsubstituted aliphatic or heteroaliphatic moiety; and

each occurrence of A is independently a carbohydrate domain of formula **(I^{det})**, **(II^{det})** or **(III^{det})**.

82. The antibody or antibody fragment of claim 80, wherein the antigen has the structure:



wherein q is 0 or 1;

each occurrence of s is independently an integer from 2-20;

t is an integer from 1-6;

R^{X1} is hydrogen, alkyl, acyl, aryl, heteroaryl, -alkyl(aryl), -alkyl(heteroaryl) or a nitrogen protecting group;

R is hydrogen or an immunogenic carrier;

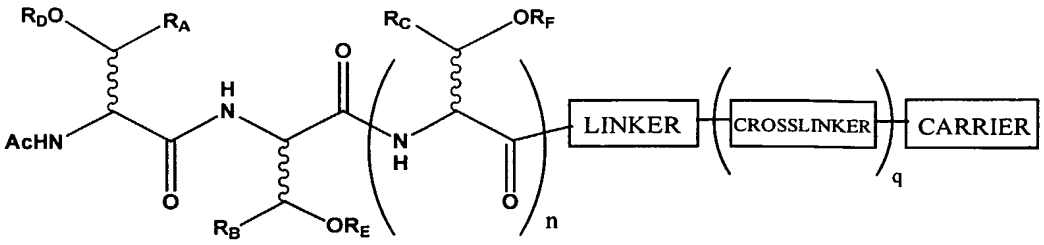
each occurrence of the spacer is independently a substituted or unsubstituted aliphatic, heteroaliphatic, aryl, heteroaryl or peptidic moiety;

the linker is either a free carboxylic acid, –O-, (carboxamido)alkyl carboxamide, MBS, primary carboxamide, mono- or dialkyl carboxamide, mono- or diarylcarboxamide, linear or branched chain (carboxy)alkyl carboxamide, linear or branched chain (alkoxycarbonyl)alkyl-carboxamide, linear or branched chain (carboxy)arylalkylcarboxamide, linear or branched chain (alkoxycarbonyl)alkylcarboxamide, an oligoester fragment comprising from 2 to about 20 hydroxy acyl residues, a peptidic fragment comprising from 2 to about 20 amino acyl residues, or a linear or branched chain alkyl or aryl carboxylic ester;

each occurrence of L^1 is independently a substituted or unsubstituted aliphatic or heteroaliphatic moiety; and

each occurrence of A is independently a carbohydrate domain of formula **(I^{det}), (II^{det})** or **(III^{det})**.

83. The antibody or antibody fragment of claim 80, wherein the antigen has the structure:



wherein the linker is -O-, -NR_G-, -$NR_G(CR_HR_J)_kNR_K$-, $NR_G(CR_HR_J)_kNR_K(C=O)(CR_HR_J)_kS$-, -$(CR_HR_J)_kNR_K$-, -$O(CR_HR_J)_kNR_K$-, an oligoester fragment comprising from 2 to about 20 hydroxy acyl residues, a peptidic fragment comprising from 2 to about 20 amino acyl residues, or a linear or branched chain alkyl or aryl carboxylic ester, wherein each occurrence of k is independently 1-5;

wherein each occurrence of R_G, R_H, R_J or R_K is independently hydrogen, a linear or branched, substituted or unsubstituted, cyclic or acyclic alkyl moiety, or a substituted or unsubstituted aryl moiety;

wherein the crosslinker is a moiety derived from a crosslinking reagent capable of

conjugating the carrier with the linker;

wherein the carrier is a peptide, protein or lipid;

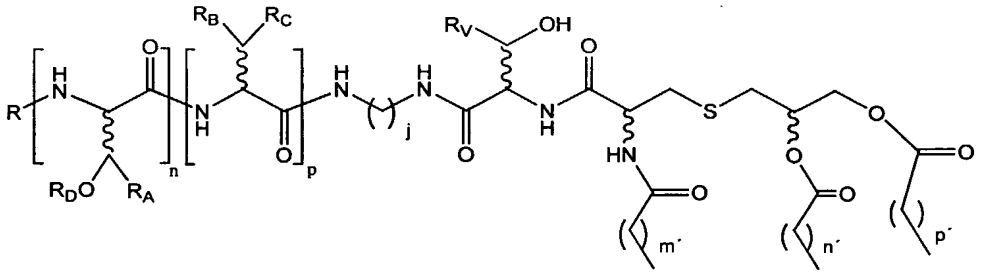
wherein n is 1, 2, 3 or 4;

wherein q is 0 or 1;

wherein each occurrence of R_A, R_B and R_C is independently hydrogen, substituted or unsubstituted linear or branched chain lower alkyl or substituted or unsubstituted phenyl; and

wherein each occurrence of R_D, R_E and R_F are each independently a carbohydrate domain of formula **(I^{det})**, **(II^{det})** or **(III^{det})**.

84. The antibody or antibody fragment of claim 80, wherein the antigen has the structure:



wherein n and p are each independently an integer from 1-6;

m′, n′ and p′ are independently integers between about 8 and 20;

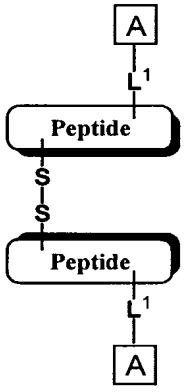
j is an integer between 1 and about 8;

R is a nitrogen protecting group;

R_V, and R_A, R_B, R_C, R_E and R_F, for each occurrence, are independently hydrogen, substituted or unsubstituted linear or branched lower alkyl or substituted or unsubstituted phenyl;

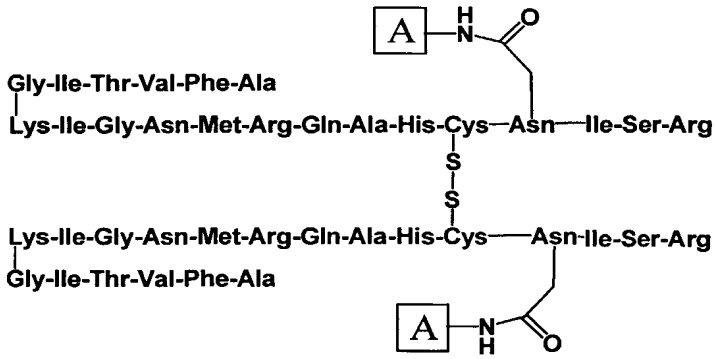
each occurrence of R_D is independently a carbohydrate domain of formula **(I^{det})**, **(II^{det})** or **(III^{det})**.

85. The antibody or antibody fragment of claim 80, wherein the antigen has the structure:

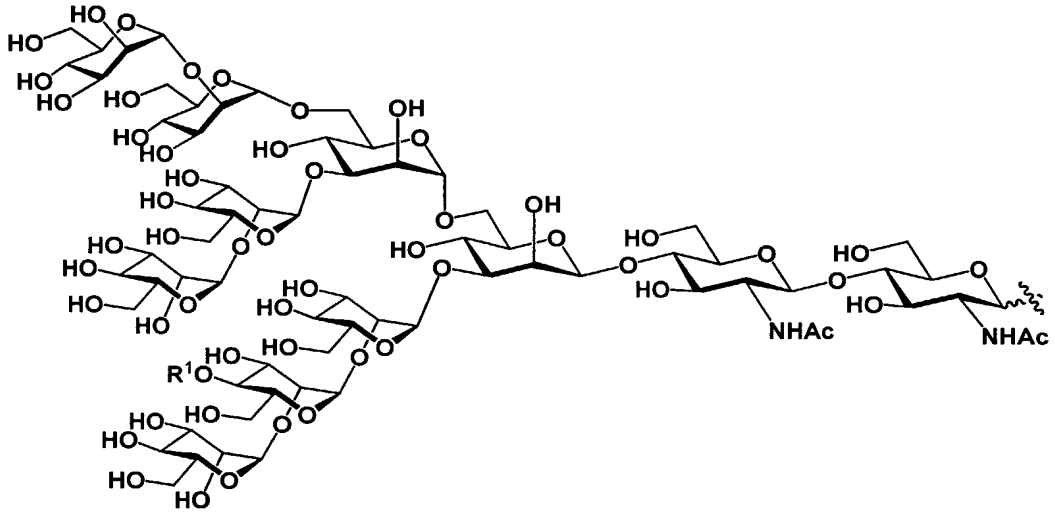


wherein each peptide may be the same or different; and each occurrence of A is independently a carbohydrate domain of formula **(I^{det})**, **(II^{det})** or **(III^{det})**.

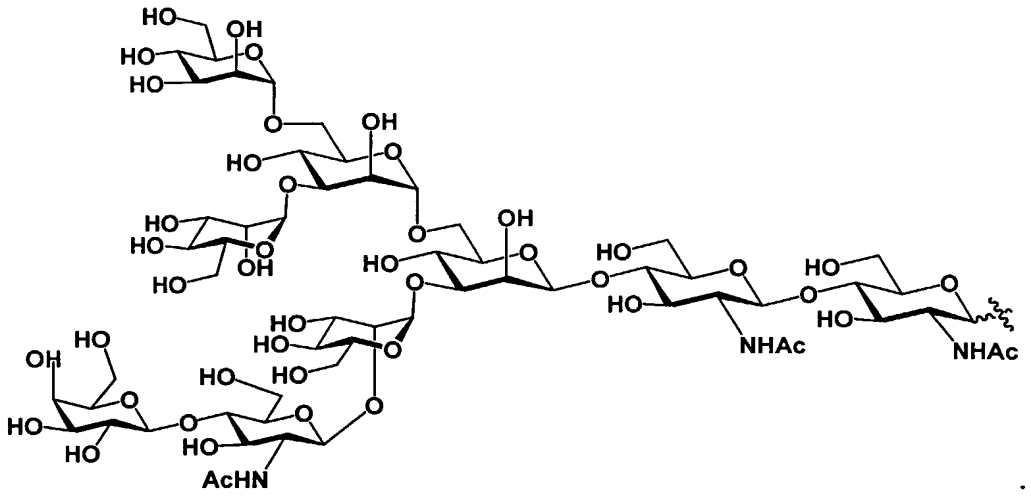
86. The antibody or antibody fragment of claim 85, wherein the antigen has the structure:



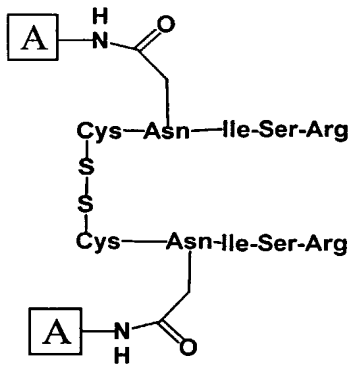
wherein each occurrence of A is independently a carbohydrate domain having one of the structures:



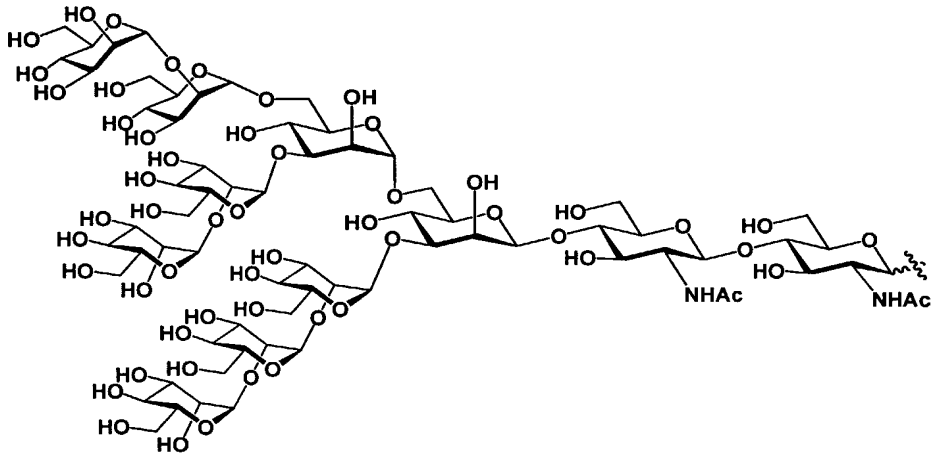
or



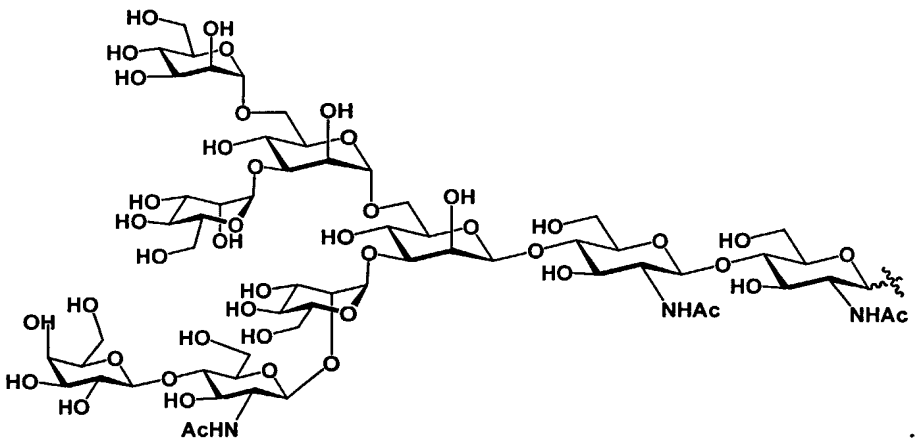
87. The antibody or antibody fragment of claim 85, wherein the antigen has the structure:



wherein each occurrence of A is independently a carbohydrate domain having one of the structures:



or



88. The antibody or antibody fragment of claim 77, wherein the antibody is a monoclonal antibody.

89. A pharmaceutical composition comprising an effective amount of a construct of claim 28 or 29; in admixture with a pharmaceutically suitable diluent or carrier.

90. A composition for eliciting an immune response in a subject comprising an effective amount of a construct of claim 28 or 29, said amount being effective to induce antibodies in a subject;

in admixture with a suitable immunogenic stimulant.

91. The composition of claim 90 wherein the immunogenic stimulant comprises *Salmonella minnesota* cells, bacille Calmette-Guerin or QS21.

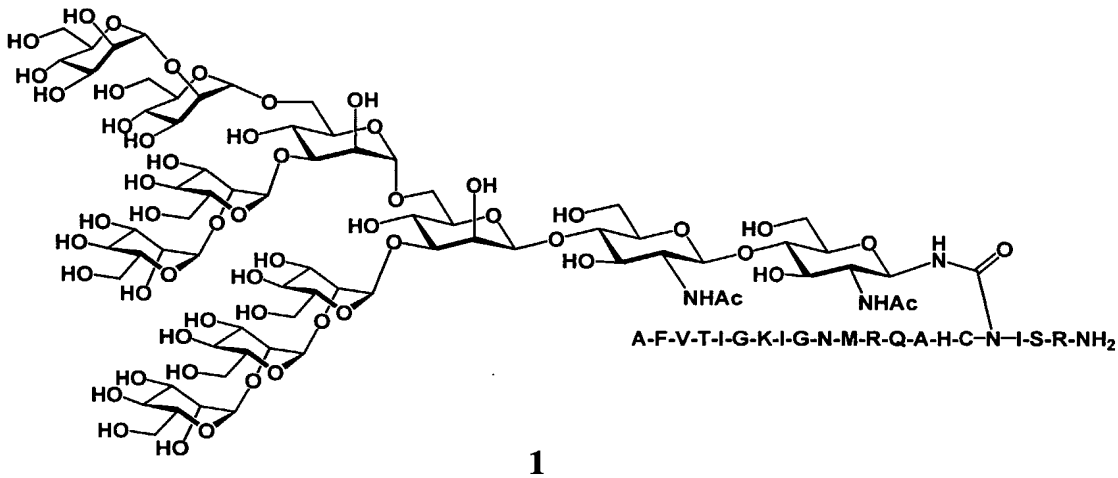
92. A method of eliciting antibodies in a subject comprising administering to the subject a composition of claim 90.

93. A method of preventing infection with HIV comprising administering to a subject an effective amount of a composition of claim 90.

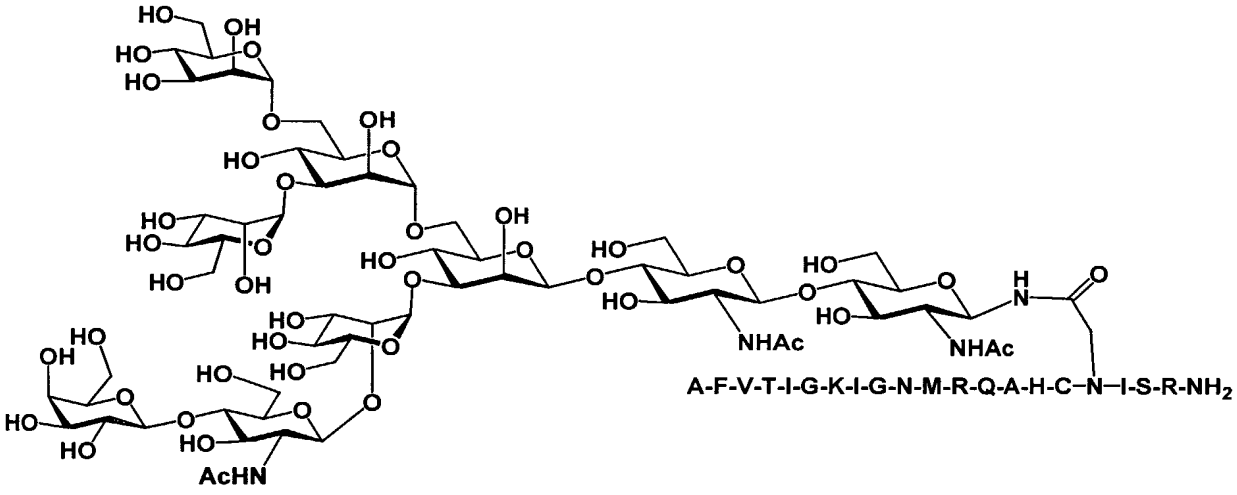
94. A method of treating an HIV infection comprising administering to a subject in need thereof an effective amount of a composition of claim 90.

95. A method of eliciting an immune response wherein the response is directed against an antigen comprising a carbohydrate epitope expressed on the surface of gp120, said antigen being a construct of claim 28 or 29.

96. The method of claim 94 further comprising administering an adjuvant.


NHAc
NHAc
A-F-V-T-I-G-K-I-G-N-M-R-Q-A-H-C-N-I-S-R-NH2

1


NHAc
NHAc
AcHN
A-F-V-T-I-G-K-I-G-N-M-R-Q-A-H-C-N-I-S-R-NH2

2

FIG. 1